Linking Community Ecology to Biodiversity Patterns

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Para Alba y Luis

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Table of Contents

Zusammenfassung

Das Verständnis der Verbreitungsmuster von Biodiversität. Das ist ein Hauptziel der Forschung zu Ökologie und Evolution. Jedoch macht die Komplexität dieses Themas es notwendig, es in vielzählige Teilbereiche aufzuspalten, wie beispielsweise die evolutionären Beziehungen zwischen Arten und die Faktoren hinter Biodiversitätsmustern in kleinem und großem Maßstab. Antworten auf diese Themen fungieren als kleine Bausteine, die Stück für Stück dazu beitragen unser Wissen und Verständnis über Biodiversität, und damit gleichzeitig über die unübersichtlichen Prozesse und Mechanismen der Aufrechterhaltung der Artenvielfalt, auf- und auszubauen. Der Beitrag dieser Forschungsarbeit hierzu ist die Studie von einigen Faktoren, welche die Muster der Pflanzendiversität auf unterschiedlichen räumlichen Maßstäben erklären.

Die Erforschung von Biodiversitätsmustern ist keinesfalls ein neues Forschungsgebiet; es ist mindestens seit Beginn des 20. Jahrhunderts gut dokumentiert. Dennoch forschen wir nach wie vor zu diesem Themengebiet, da ein generelles Fazit bis heute außer Reichweite liegt. Die ersten Biologen, die sich der Muster der Biodiversität annahmen, taten dies, weil sie die großen Unterschiede in der Ausbreitung von Arten in verschiedenen Lebensräumen beobachteten. Diese Beobachtungen führten zur Entstehung diverser Forschungsgebiete, wie Ökologie oder Biogeographie. Mit der Zeit haben diese verschiedenen Forschungsgebiete einige der Hauptmechanismen aufgedeckt, die für das Auftreten von Arten in speziellen Lebensräumen verantwortlich sind, wie zum Beispiel biologische Nischen. Dennoch bleiben viele Beziehungen wenig verstanden. So wissen wir zwar, dass klimatische Bedingungen eine wichtige Rolle in der Entstehung von Biodiversitätsmustern spielen, doch wie wichtig ist diese Rolle im Vergleich zu anderen Faktoren, wie die Ausbreitungskapazität der Art oder das Vorkommen anderer Arten?

Jedes Kapitel dieser Arbeit befasst sich mit dem Thema der Diversitätsmuster von Pflanzen und deren Ursachen aus einem anderen Blickwinkel heraus. Konstant bleiben dabei die zwei Hauptfragen: i) Wie viele Pflanzenarten können in einer Gemeinschaft koexistieren? Und ii) was bestimmt, welche Arten in einem Gebiet auf unterschiedlichen räumlichen Maßstäben koexistierten können? Kapitel 1 zielt speziell auf die Fragestellung ab, ob es eine Obergrenze an koexistierenden Arten in einer Gemeinschaft gibt.

Dafür habe ich die Literatur über die Definitionen von ‚Sättigung der Artenzahl' und ‚beschränkte Diversität' aufgearbeitet. Diese Literaturrecherche ergab, dass es keine offizielle Definition zu 'Sättigung der Arten' gibt, weshalb ich eine allgemeine Definition vorschlug. Ich kam zu der Schlussfolgerung, dass eine Sättigung vorliegen oder nicht vorliegen kann, je nach dem welches System betrachtet wird. Um Verwechslungen vorzubeugen und vergleichbare Ergebnisse in zukünftigen Studien zu ermöglichen, habe ich daher eine Liste von Vorgaben erstellt, welche erfüllt werden müssen, um eine Artensättigung nachzuweisen. In Kapitel 2 versuche ich darzulegen, was die Variation in der Artenzusammensetzung auf verschiedenen räumlichen Maßstäben bestimmt. Im Besondern habe ich die relative Bedeutung von Artenvielfalt, Klima und geographische Distanz als Bestimmungsgrößen für den Wechsel (Beta-Diversität) von Farnarten auf und zwischen lokaler und

regionaler Ebene betrachtet. Die Ergebnisse betonen die Dominanz von a) regionalem Klima auf den *lokalen* Artenwechsel und b) lokale Artenvielfalt auf den *regionalen* Artenwechsel.

In Kapitel 3 gehe ich das Gebiet der Diversitäts-Muster von einer sehr lokalen Ebene an, indem ich die Auftrennung von Arten in einer hoch-diversen epiphytischen Gesellschaft untersuche. In diesem Kapitel komme ich zu dem Schluss, dass auch in einem Lebensraum mit einer geringen Artenzahl sich die Arten offenbar als Reaktion auf das Auftreten anderer Arten aufspalten. Letztendlich gewinne ich in Kapitel 4 etwas Abstand und teste, ob die lokalen Vorkommen einer der diversesten pflanzlichen Abstammungslinien der Erde („Grammitid-farne“) gleiche Artenanzahlen über geographisch- und evolutionär unabhängige Areale aufweisen. Mit diesem Test beabsichtige ich eine sehr vorläufige Einschätzung der Obergrenzen für die Anzahl von koexistierenden Arten und somit Artensättigung zu erhalten. Ich folgere aus meiner Forschung, dass vorläufige Anzeichen existieren, die das Auftreten von Artensättigung innerhalb dieser Gemeinschaften belegen und dass weitere Forschungsarbeiten robustere Schlussfolgerungen erzielen könnten, indem sie Nischenanalysen und den phylogenetischen Hintergrund der Arten mit einbeziehen. Dies war leider im Rahmen meiner Dissertation nicht mehr möglich.

Synopsis

Understanding the distribution patterns of biodiversity. This is a primary goal of studies on ecology and evolution, but the complexity of this subject makes it necessary to divide it into numerous narrow topics, e.g., the evolutionary relationships between species and the determinants of biodiversity patterns at large and small scales. Answers to these topics act as little bricks, each contributing to build and give structure to our knowledge of biodiversity and its intricate processes and mechanisms of maintenance. The contribution of this thesis is the study of some of the factors that explain the patterns of plant biodiversity at different spatial scales.

The study of biodiversity patterns is by no means a novel topic, it has been documented in the scientific literature at least since the beginning of the 20th century. Yet, we are still studying it because we are far from reaching a general conclusion. The first biologists who approached the patterns of biodiversity did so because they observed the large differences in the distribution of species among different habitat types. These observations initiated different fields of research like ecology and biogeography. Over time, these disciplines have unravelled some of the main mechanisms that determine the occurrence of species in particular areas, one of the most important being the discovery of biological niches. However, many relationships are still poorly understood. For instance, we know that climatic conditions play an important role in determining biodiversity patterns, but how important is this role when compared to other factors like the dispersal capacity of species or the presence of other species?

Each chapter in this manuscript approaches the topic of plant diversity patterns and its determinants from a different perspective but in response to one of these main questions: i) how many species can coexist in a given assemblage? and ii) what determines which species coexist in areas at different spatial scales? Chapter 1 aims specifically to answer the question: are there limits to the number of species that can coexist in a given assemblage? Here, I reviewed the literature searching for the definition given to species saturation or bounded diversity. With this search, I determined that a formal definition of species saturation did not exist and therefore I suggested a general definition. I concluded that saturation can or cannot be found depending on the study system. Thus, to avoid confusion and obtain comparable results in future studies, I provided a list of requirements needed to demonstrate species saturation. In Chapter 2 I aimed to understand what determines the variation in species composition at different spatial scales. Specifically, I studied the relative importance of species diversity, climate and geographical distance as determinants of the turnover (β diversity) of fern species at local and regional scales and across them. The results highlight the governance of a) regional climate on the local turnover of species and b) local species diversity on the regional turnover.

In Chapter 3, I tackled the subject of diversity patterns from a very local scale by studying the segregation of species in a highly diverse epiphytic assemblage. In this Chapter, I concluded that even in a habitat with a low density of species, they segregate apparently in response to the presence of other species. Finally, in Chapter 4 I zoomed out and tested if the local assemblages of one of the most diverse lineages on Earth (grammitid ferns) have a similar number of species across geographically and evolutionarily independent areas. With this, I intended to gather very preliminary evidence of the

limits to the number of coexisting species or saturation. I concluded that preliminary evidence exists for the occurrence of species saturation within these assemblages and that further studies could reach more robust conclusions by incorporating niche analyses and the phylogenetic background that was beyond my PhD studies.

Introduction

In this introduction to the thesis, I provide a brief background to three subjects that are fundamental to the understanding of biodiversity patterns: limits to biodiversity, β diversity and niche. The general aim and fieldwork campaign of the thesis are presented and a summary of each chapter is subsequently given, the first chapter is a literature review and the final three chapters are primary research.

a. Limits to biodiversity

The increase in the number of species of a lineage is a process known as radiation (Linder 2008). Among different types including ancient and geographic radiations, adaptive radiations are those where the number of species increases in response to certain environmental conditions (Givnish & Sytsma 2000; Schluter 2000). The results from studies on adaptive radiations have shown that in the initial phase of a radiation the evolution of species occurs very rapidly relative to geological time, or in other words, the rates of diversification are high (MacArthur 1965; Walker & Valentine 1984; Gavrilets & Vose 2005). In a later phase, the initial high rates of diversification decline slowly, as if something had put a break in the evolutionary machine, and thus species within the clade keep emerging but at a slower rate because the extinction of other species increases (MacArthur 1965; Schluter 2000; Gavrilets & Vose 2005; Moen & Morlon 2014). In some cases, the diversification rate ceases, which does not mean that the clade stops producing new species but that the emergence of new species is apparently compensated with the extinction of others (Walker & Valentine 1984; Jablonski & Sepkoski 1996).

A fascinating example of an adaptive radiation is the diversification of *Anolis* lizards, a highly diverse genus of the Caribbean islands. *Anolis* species adapted to a range of habitats that vary from ground-dwelling species to those inhabiting specific zones of the trunk or branches of the trees (Losos 1994). This lineage, however, has shown a declining rate of diversification since a few millions of years ago in three of the islands where it occurs. Mahler *et al.* (2010) and Rabosky & Glor (2010) have shown that this decline is proportional to the island's area. A central and intriguing implication of these findings is the possibility that diversification is limited by certain ecological constraints.

The causes of a decelerated diversification are still as intriguing as those that allow the initial increase. A long-standing hypothesis states that radiations may be limited by the number of species that can co-occur in each environment (Ricklefs 1987a; McPeek 2008), both in local assemblages and within regional biomes. This limitation is in turn determined by processes at different scales of space and time. For instance, while area and climate are the main abiotic predictors of speciation rates at regional scales (Rosenzweig 1995; Chown & Gaston 2000; although see Linder 2008), geological and biogeographical effects shape diversity patterns at all scales because they change the distribution of environmental conditions through time (Cowling & Lombard 2002; Hoorn *et al.* 2010; Antonelli 2015).

Thus, understanding the patterns of biodiversity requires the integration of the macroevolutionary context that includes the processes generating the species being assembled (Ricklefs 1987b, a; Ricklefs *et al.* 1999; Webb *et al.* 2002; McPeek 2008). But the integration of patterns and relationships at small scales is also relevant because it is at these scales where the organisms

interact and the whole biological machinery that triggers e.g. genes and adaptations is at work. It follows then that an integration of community ecology in studies of species diversification is an important challenge that provides novel perspectives to the understanding of biodiversity patterns, Chapters 1 and 4 present some of the aspects to be considered in this integration.

b. Species turnover or β diversity

From an ecological perspective, one of the indicators that allows us to observe and understand the relationships between biodiversity patterns at different spatial scales is the turnover of species, i.e., the change in the identity of species between areas also known as β diversity (Whittaker 1960, 1972). β diversity provides a different level of information than counts of species numbers (α and γ diversity) because it informs on their identity. β diversity reflects the variation in species arrangements and it, therefore, sheds light on the processes and mechanisms responsible for regional biodiversity patterns (Williams 1996; Moritz *et al.* 2001). Areas with high β diversity typically have small, patchily distributed species populations, and areas with low β diversity usually have widespread species and are rather homogeneous in terms of species composition. An analysis of the distribution of β diversity in the Americas revealed that opposite to α and γ diversity, whose most general distribution follows a latitudinal gradient, β diversity varies along gradients of habitat complexity, for instance along elevational gradients (McKnight *et al.* 2007).

Mountain areas hold higher β diversity values than the lowlands as a result of high environmental heterogeneity along relatively small geographical distances. Environmental heterogeneity makes thus mountain ecosystems vital for the generation and maintenance of biodiversity because they offer a wealth of opportunities for the adaptation and diversification of species (Fjeldså et al. 2012, Wang et al. 2012, Hoorn et al. 2013, Hughes and Atchison 2015, Antonelli 2015). Regarding the determinants of β diversity, studies in the lowland areas of South America have revealed geographical distance, as a proxy for dispersal capacity, as the most influential factor determining β diversity (Tuomisto *et al.* 1995, 2003, Kristiansen *et al.* 2011, 2012; Zuquim *et al.* 2012). In the mountains, however, dispersal capacity becomes less relevant because habitats' extensions are smaller. Instead, a high spatial variability of habitat conditions related to topographical factors such as elevation, aspect, inclination, and landscape arrangement determine β diversity patterns in the highlands (Kessler, 2000; Kessler et al., 2001; McKnight et al., 2007; Mourelle & Ezcurra, 1997; Tello et al., 2015). Finally, a less understood aspect of β diversity studies is how processes acting at one spatial scale (e.g. local scales) influence β diversity patterns at another scale (e.g. regional scale), this is the main topic of Chapter 2.

c. Niche

Environmental characteristics are of utmost importance in determining biodiversity patterns. These characteristics can be classified in two coarse groups, abiotic that includes components like latitude, soils and topography, and biotic that includes all interactions between species, which constitutes an active part of the surrounding environment.

'Niche' was the term used to describe the resource requirements of a species in a given habitat (Grinnell 1917). The fact that different species have different requirements or niches means that species occupy different positions in the community (Elton 1927). Volterra (1926) and Gause (1934 a,b) translated these observations and assumptions into the principle of competitive exclusion, whereby two species cannot coexist if they depend exactly on the same resources, and therefore, species should specialize in the consumption of different resources to avoid competition with other species. Since each species requires different kinds of resources for their survival, growth and reproduction, Hutchinson (1957) proposed that each niche is a multidimensional space characterised by several axes, one for each specific requirement.

Understanding plants coexistence under niche hypotheses is challenging because most plants have similar resource requirements, like soil nutrients, water, light and CO_2 (Silvertown 2004). Still, previous studies suggest that niche segregation and competition are of major importance for the structure and dynamics of plant assemblages (Goldberg & Barton 1992; Tilman 1994; Wright 2002). One mechanism by which plants define their particular niche is by differing in the optimal environmental conditions at which their physiological activities work best (Austin 1985). Thus, once the large and medium scale climatic and biogeographical constraints are considered, the distribution of species is further refined at local spatial scales (Fukami 2015). Finally, the location of each species is thus defined by its functional relationship to the environment and in relation to the requirements of other coexisting species.

Occupying different niches must be a challenge among groups of closely related plant species which frequently have similar morphological and physiological constraints. This is the reason for the hypothesis that competition increases with the degree of relatedness among species (Tilman & Pacala 1993; Givnish 2010). This hypothesis also implies that intraspecific competition is stronger than between different species (Tilman 1982, 1990). Empirical tests for the occurrence of these competitive interactions in plant communities should be possible because if competition exists, then the increase in the number of species should tend to segregate species in particular arrangements and stabilize or reduce the number of individuals of other species in the community. Preliminary attempts to these tests are presented in Chapters 3 and 4.

d. Ferns as a research group

Ferns are among the most numerous and widespread plants on Earth. Their first ancestors originated 400 Myr ago and most groups diversified during the Cenozoic ca. 70 Myr ago (Moran 2004, Testo & Sundue 2016). Currently, more than 11,000 species of ferns are recognized, and this number increases every year because more species are being discovered. Ferns are main contributors to the terrestrial and epiphytic flora (Galeano *et al.* 1998; Zotz 2005). They have developed a remarkable diversity of growth forms, divided among 8500 terrestrial species and 2865 epiphytes worldwide (Zotz 2013). Ferns are globally distributed, although most of their diversity is found in the tropics and more specifically in the wet tropical mountains (Kessler *et al*. 2011). Drought is a major limitation to ferns' survival because of their low physiological water use efficiency (Brodribb *et al.* 2005; Brodribb & McAdam 2011), nevertheless, 17 fern species occur in the mountain ranges of the Sahara desert (Anthelme *et al.* 2011).

Another example of their extreme habitats are some members of the grammitid group that have been reported in the freezing Andean mountains at 4550 m, which makes them the epiphytes reaching the highest elevation in the world (Sylvester *et al.* 2014).

Their diversity and relatively well resolved taxonomy have made ferns an ideal group for studies on tropical biodiversity (e.g.,Tuomisto *et al.* 1995; Kessler *et al.* 2001; Karger *et al.* 2014), elevational patterns of diversity (Kluge *et al.* 2006; Kessler *et al.* 2011) and soil influence on vegetation structure (e.g., Tuomisto & Ruokolainen 1994; Zuquim *et al.* 2012)

e. Aim

In general, this thesis aims to understand some of the factors that explain the patterns of plant biodiversity at different spatial scales. Each of the chapters in this manuscript approaches the topic of plant diversity patterns and its determinants in response to one of these main questions: i) how many species can coexist in a given assemblage? and ii) what determines which species coexist in areas at different spatial scales?

f. Fieldwork campaign

Figure 1 shows the global distribution of 1730 plots included in this thesis where 1196 terrestrial and epiphytic fern species were recorded. The field data used in this thesis come from different sources. Fern plots-data have been gathered over 20 years by Michael Kessler's group and these currently cover all major areas of distribution. From 1995 to 1997 he and his collaborators sampled more than 1200 plots along the Bolivian Andes and I used these data to test beta-diversity hypotheses in Chapter 2. Additionally, from 2000 to 2016 several students from this group conducted fieldwork in America, Africa and Asia. Data from 1090 plots from the three continents have been included in Chapter 4 for comparison between grammitid-fern assemblages.

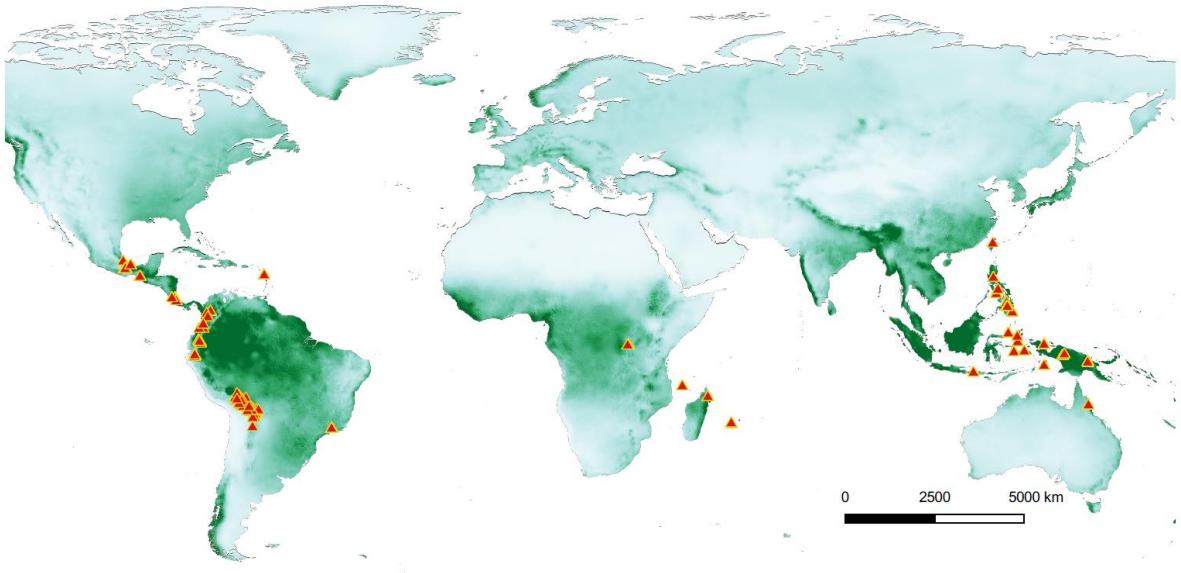

Figure 1. General distribution of plots across America (1380 plots), Africa (43 plots) and Asia (307 plots).

Sampling sites covered different elevational ranges in each country from the lowest to the highest forest areas available. Plots consisted of 400 m^2 areas (usually 20 m x 20 m) where the presence or abundance of either all fern species or some targeted taxa was recorded. Environmental information recorded included elevation, topography, aspect, epiphytic moss cover as a proxy for air humidity (Karger et al., 2012), and canopy openness.

From all plots included in this thesis, I specifically sampled 150 plots along the Andean mountains in Costa Rica, Colombia and Ecuador. The tropical Andes cover 3300 km in length and reach up to 6768 m in elevation. These mountains encompass the amazing variety of tropical ecosystems from dry lowlands to glaciers. It is therefore not strange that shortly after the uplift of the Andes numerous radiations of plants and animals have taken place, turning these mountains into one of the richest places on Earth. The tropical Andes are recognised as biodiversity hotspots (Myers et al., 2000) and therefore an ideal place to study coexistence and biodiversity patterns.

g. Chapters overview

In this section, I present the most important results of each chapter using graphical abstracts.

Chapter 1: Assessing species saturation: conceptual and methodological challenges

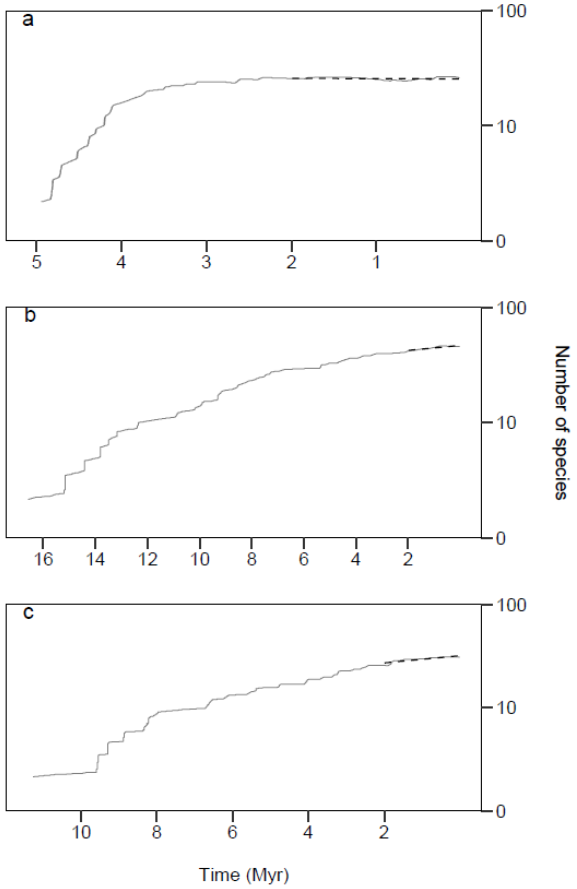

Figure 2. Number of species through time for *Dendroica* (a), *Heliconius* (b) and *Plethodon* (c) (Modified after Etienne *et al.* 2011). Linear models demonstrate saturation for the warbler genus *Dendroica* during the last two Myr (R^2=0.016, F=4.434, p-value=0.04). The butterflies' genus *Heliconius* and the salamander genus *Plethodon*, show an ongoing increasing trend during the same period (R^2=0.845, F=308.4, p-value < $2.2e^{-16}$; R^2=0.798, F=357.4, p-value < $2.2e^{-16}$, respectively).

This chapter is a literature review of the concept of species saturation with the aim to investigate how saturation is defined in biological systems, and how it can be demonstrated. Studies on saturation have attempted to answer the question: is there a maximum number of species that can coexist? Patterns of diversification through time like the ones shown in Fig. 2 suggest that there is an upper limit to the that a lineage can produce, but defining and testing this has proven problematic. In this review, I recommend the following definition of saturation: *saturation is the dynamic equilibrium of species richness due to niche limitation within a given group of organisms and within an environmental and temporal reference frame*. A discussion on the general challenges and methodological approaches of studies on species saturation is presented. This chapter has been accepted for publication in a peer-reviewed journal: I. Olivares, Dirk N. Karger, M. Kessler in *Biological Reviews* (in press).

Chapter 2: Determinants of beta diversity within and across different spatial scales in mountain ecosystems

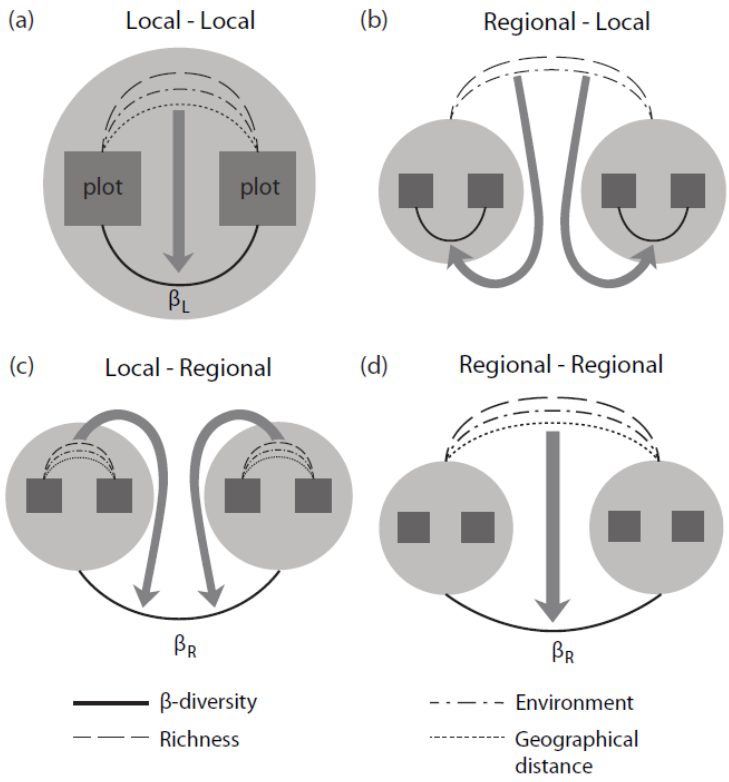

Figure 3. Four combinations of scales that may influence measures of β diversity and the hypotheses tested in Chapter 2. I used plots (squares) as sampling units at the local scale, and sites (circles) as sampling units at the regional scale. At each scale, I calculated Euclidean dissimilarity matrices in species composition (β diversity), species richness, and environmental and geographical distances. Four main types of relationships were assessed, (a) and (d) test for single-scale relationships: (a) the influence of between-plot variation in species richness, environment, and geographical distance on local β diversity; and (d) the same at the regional scale. (b-c) test for cross-scale relationships: (b) the influence of regional factors (except distance) on local β diversity, and (c) the influence of local factors on β_R.

In chapter 2, I explored how local and regional factors influence β diversity at each scale, and across them. The results from this chapter contrast with those from nearby lowland regions because they highlight the importance of environmental heterogeneity on the distribution and evolution of biodiversity in mountain ecosystems. This chapter is under review in a peer-reviewed journal: I. Olivares, Michael Kessler. Submitted to Global Ecology and Biogeography on 29.12.2017.

Chapter 3: *Ascogrammitis lehnertii* (Polypodiaceae): A new species from a species-rich community of grammitid ferns in the Andes of Ecuador

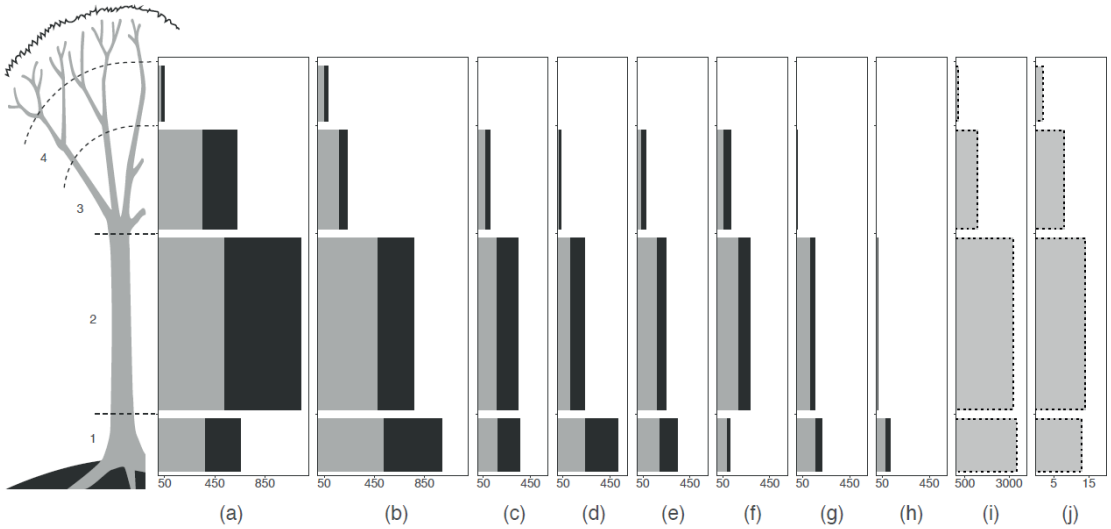

Figure 4. Vertical distribution of the eight most abundant grammitid species on Cerro Toledo (Ecuador) following the vertical zonation of Johansson with zone 1 corresponding to the trunk bases, zone 2 to the trunks, zone 3 to the main branches, and zone 4 to the canopy branches. Bars indicate the number of sterile (gray) and fertile (black) individuals per zone. The two rightmost panels show the total numbers of individuals (i) and species (j) per zone. (a) *Stenogrammitis jamesonii*, (b) *Ascogrammitis lehnertii*, (c) *Mycopteris leucosticta*, (d) *Lellingeria pseudocapillaris*, (e) *Melpomene sodiroi*, (f) *Moranopteris aphelolepis*, (g) *Melpomene cf. personata*, (h) *Alansmia.*

Aiming to understand how diversity is maintained at the local scales, over the last few years I conducted detailed field surveys in biodiversity hotspots in South and Mesoamerica. The Amotape-Huncabamba Zone between southern Ecuador and northern Peru is one of these regions because it acts as a species corridor allowing for interchange between the northern and central Andean species, and the elevational depression of the zone also allows for interchange between the western and eastern cordilleras. As a result of these field surveys, I collected a new epiphyte fern species and reported a population of another species previously restricted to the Ecuadorian Chocó. This chapter describes these discoveries and characterizes the grammitid assemblage in the light of the habitat segregation strategies that might facilitate the species coexistence in such diverse areas. Fig. 4 shows the distribution of the eight most common species in the community, which segregate by habitat into trunk-base and trunk specialists. This chapter has been accepted in a peer-reviewed journal: Michael Sundue, I. Olivares, Michael Kessler in *Systematic Botany* (in press).

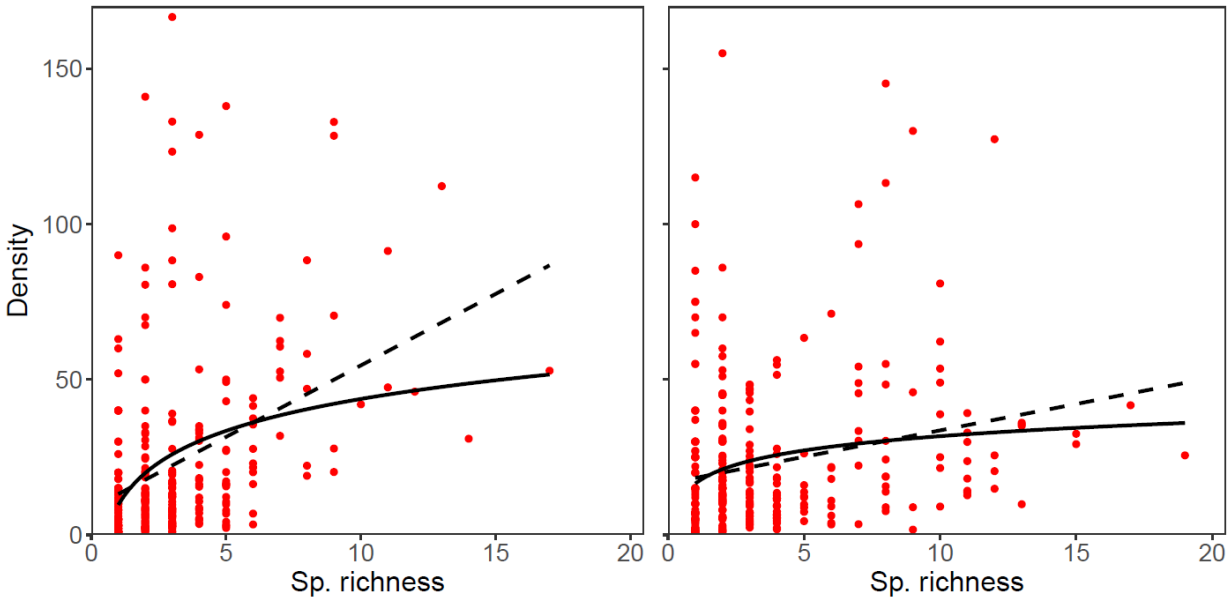

Figure 5. Relationship between the average number of individuals per species per plot (density) versus the total number of grammitid species per plot (richness) in 296 plots in America (a) and 307 plots in Asia (b). The logarithmic lines are better fits to the empirical data than the linear relationships, indicating niche limitation. Left panel: America, linear model: AIC=1353.368; Adj R2= 0.057; p-value= 1.737e-05; log model: AIC=602.368; Adj R2=0.0634; p-value= 6.893e-06. Right panel: Asia, linear model: AIC=1605.941, Adj R2= 0.0331; p-value= 0.0008; log model: AIC= 733.773; Adj R2= 0.025; p-value= 0.00325.

In this last chapter, I explored some of the questions and hypotheses suggested for saturation studies in Chapter 1. Here, I specifically asked if there is any evidence of stability and niche limitation in grammitid assemblages in three geographically independent regions in America, Africa and Asia. To do this I analysed the composition of grammitid communities and tested if (a) there are differences in the distribution of grammitid species richness along the climatic gradients in these three regions, and (b) if there is any evidence of density dependence as a proxy for niche limitation, specifically if the number of species or their number of individuals decreases with an increasing number of species in the assemblage. These questions will provide evidence for the hypothesis that species composition in such assemblages is governed by a limited number of species that can enter them.

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Chapter 1

Assessing Species Saturation: Conceptual and Methodological Challenges

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Assessing species saturation: conceptual and methodological challenges

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ABSTRACT

Is there a maximum number of species that can coexist? Intuitively, we assume an upper limit to the number of species in a given assemblage, or that a lineage can produce, but defining and testing this limit has proven problematic. Herein, we first outline seven general challenges of studies on species saturation, most of which are independent of the actual method used to assess saturation. Among these are the challenge of defining saturation conceptually and operationally, the importance of setting an appropriate referential system, and the need to discriminate among patterns, processes and mechanisms. Second, we list and discuss the methodological approaches that have been used to study species saturation. These approaches vary in time and spatial scales, and in the variables and assumptions needed to assess saturation. We argue that assessing species saturation is possible, but that many studies conducted to date have conceptual and methodological flaws that prevent us from currently attaining a good idea of the occurrence of species saturation.

Key words: biodiversity, niche, community assembly, carrying capacity, equilibrium, species– area, stability, immigration– extinction, species pool.

CONTENTS

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I. INTRODUCTION

Is there a maximum number of species that can coexist in a given assemblage? And is there a maximum number of species that a lineage can produce? These questions have puzzled biologists for decades because they have important implications for understanding how biological diversity is spatially distributed and evolutionarily regulated. In effect, ecological and evolutionary theory predicts that an upper limit must exist (Schluter, 2000; Ricklefs, 2006; Phillimore & Price, 2008; Gascuel *et al.*, 2015; Rabosky & Hurlbert, 2015). However, the question is not so much about whether a limit as such exists, but rather whether it has been reached. Numerous studies have attempted to address this question, but their answers differ: many studies support the idea of saturation (e.g. Terborgh & Faaborg, 1980; Sepkoski, 1984; Alroy, 2008; Rabosky & Lovette, 2008; Rabosky & Hurlbert, 2015) while many others reject it (e.g. Benton, 1995; Cornell, 1999; Stohlgren *et al.*, 2008*a*; Harmon & Harrison, 2015), and some others explain its relativity with regard to different factors like spatial scale (Shurin *et al.*, 2000), seasonality (Starzomski, Parker & Srivastava, 2008), and species interactions (Cornell & Lawton, 1992; Stachowicz & Tilman, 2004). Often, answers are based on different definitions of saturation or the same definitions are tested at different spatial or temporal scales, which prevents reaching any definite conclusion about the prevalence of saturation. The lack of a standard definition or classification limits our ability to test appropriately for saturation in ecological and evolutionary contexts. Thus, despite the apparent simplicity and intuitive appeal of the concept, testing for saturation has proved problematic, to the degree that its appropriateness as a concept has been questioned by some researchers who claimed that it might lead to confusing ideas in ecology (Whittaker, 1977). Terborgh & Faaborg (1980, p. 178) wrote ‘‘ … no author has proposed a set of operational criteria by which a saturated community could be distinguished from an unsaturated one’’. Nearly 40 years later we still lack them.

Herein, we review the literature associated with the concept of saturation. We searched *Web of Science* and *Google Scholar* for combinations of terms related to saturation (community, niche and species saturation) and found 42 matching studies. Almost no study provided a conceptual definition of the term. Furthermore, these studies supported the comments of numerous authors that our understanding of the topic is hampered by misconceptions (Loreau, 2000), lack of a clear analytical framework (Cornell & Lawton, 1992; Cornell, 1999; Russell *et al.*, 2006), and lack of robust methodological approaches (Cresswell, Vidal Martinez & Crichton, 1995; Palmer & van der Maarel, 1995; Mateo, Mokany & Guisan, 2017), for which the cited studies suggest relevant solutions. In addition, it became evident that saturation is a phenomenon that has been assessed at different scales, from local assemblages at ecological timescales (e.g. Fox, McGrady-Steed & Petchey, 2000; Krasnov *et al.*, 2006), to entire lineages over geological periods (e.g. Brayard *et al.*, 2009; Benson *et al.*, 2014), or a combination of both (e.g. Jousselin *et al.*, 2008; Pinto-Sanchez, Crawford & Wiens, 2014; Gascuel *et al.*, 2015). Thus, in addition to the 42 matching studies, we reviewed another 101 publications that did not appear in our initial search but whose results are relevant to this review. These include studies on bounded–unbounded diversity and ecological equilibria.

Based on the conceptual background from our survey, we propose the following definition of saturation: *Saturation is the dynamic equilibrium of species richness due to niche limitation within a given group of organisms and within an environmental and temporal reference frame*.

Based on this definition, and as we explain further below, saturation can be demonstrated when we have evidence for stability and/or equilibrium of species numbers as well as evidence for niche limitation. While the restrictions of this definition might appear narrow, we argue that this avoids the interchangeable and misleading use of the terms stability and equilibrium as synonyms of saturation. According to this definition, the context matters, making it impossible to provide a more detailed definition that applies to all relevant situations because as illustrated in this review, the maximum number of species that can coexist depends on where, when, and of whom. This does not mean that for a specific study this definition should not be detailed to fit the purpose of the study.

The insights gained from our survey are presented in two parts. First, we outline seven general challenges of studies on saturation. Second, we provide a list of methodological approaches that have been used to study different aspects of saturation. These are classified in terms of what they actually test for: stability, equilibrium, or niche limitation. We explain the rationale, provide examples, and discuss the approach in the light of the seven main challenges outlined and in relation to method-specific challenges. We place special emphasis on assessing stability because this is mainly addressed in the context of studying saturation, whereas equilibrium and especially niche limitation are widely considered in ecological and evolutionary studies.

While this review is not intended to be the final word on the topic, we hope that we can add some clarity to the discussion by addressing the often-underestimated challenges of studying species saturation and summarizing the ways in which the topic has been approached.

II. SEVEN CHALLENGES IN ASSESSING SATURATION

We now address seven central issues that emerged from our review of the literature and that we consider to be of crucial importance when assessing species saturation.

(1) Conceptual and operational definitions of saturation

The use of the term saturation has varied over time and with different authors. It has its origin in chemistry, where it describes the maximum amount of solute that can possibly be dissolved in a solvent under certain conditions of temperature and pressure (Encyclopaedia Britannica, 2011). The concept of saturation as a maximum amount of species coexisting in a given region dates back to the early 20th century. Grinnell (1914, pp. 98 – 99) in his study of the animals and birds of the lower Colorado Valley asks: ‘‘is the Colorado fauna full? Are all the ecological niches, which are available in this area and which have occupants in other regions, occupied here?’’. Although Grinnell pioneered this concept, he did not coin the word saturation, and it was not until the mid-20th century that the term saturation reached a broader application in the field of ecology (Schoener, 1989).

Elton (1950, p. 20) compared the number of animal species at different sites and argued that ‘‘the number of animal [species] that can live together in an area of uniform type rapidly reaches a saturation point’’. He went on to generalize the idea (p. 22) as ‘‘in any fairly limited area only a fraction of the forms that could theoretically do so form a community at any one time.’’ Elton (p. 17) warned about the difficulty of defining the ‘‘area of uniform type’’, as he explained, ‘‘a study carried out on the animal community of a single major habitat creates, for working purposes, an arbitrary boundary that does not exist in nature’’. Finally, he also explicitly stated that it was the total number of species in a certain guild that has an upper limit, but that composition could be highly variable among assemblages (or communities as he called them) and also within assemblages through time.

A decade later, the mathematical concepts provided by the theory of island biogeography (MacArthur & Wilson, 1967) further contributed to understanding the differences in the number of species among assemblages. MacArthur & Wilson (1967, p. 176) defined saturation as the ‘‘equilibrium state’’. They proposed that as an area accumulates species the immigration rate falls, and the extinction of the species already present rises. When the extinction rate equals the immigration rate, the number of species is at equilibrium or

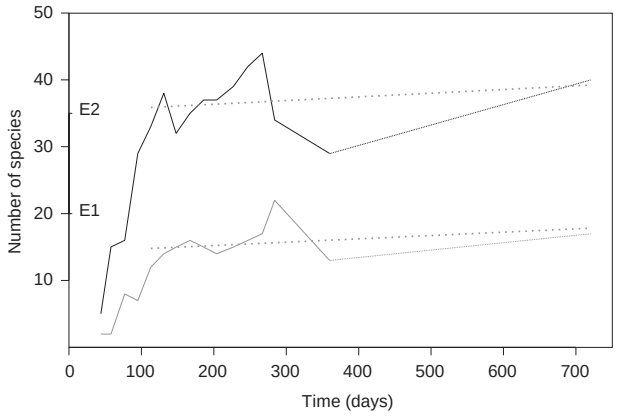

Fig. 1. A classic example of an experimental test of saturation (modified from Simberloff & Wilson, 1970). In this study, small islands (E1, E2) were defaunated and the recovery of insect richness was monitored for 2 years. Pre-defaunation number of species are indicated for each island at time zero. Linear regression (dotted grey lines) indicate no significant change in the number of species after 113 days (E1: r^2 0.01, F 1.14, P 0.31; E2: r^2 0.05, F 0.45, P 0.52). The fact that insect richness returned to pre-defaunation levels and that it then remained stable over time was taken as indication of saturation. The trajectory between years 1 and 2 is represented by a dashed line because no intermediate censuses were made.

saturated. It is important to note that the authors treated the terms equilibrium and saturation as equivalent. Later, Simberloff & Wilson (1969, 1970) carried out empirical tests of this theory, in which all insects were removed from small mangrove islands in the Florida Keys. They found that over time insect species richness returned to pre-defaunation levels on most of the islands (Fig. 1).

These examples already exemplify one of the main challenges in assessing saturation. Whereas some authors (in fact, very few) define saturation in a conceptual way, the majority of definitions are operational, in the sense that they claim that saturation can be detected when a certain number of conditions are met, such as in the above example by MacArthur & Wilson (1967). It is important, however, to realize that an operational definition is always based on a conceptual definition, even if the latter is not explicitly stated. Because the validity of any conclusions drawn from a study can only be evaluated against a set of definitions, we consider it imperative for authors to provide both conceptual and operational definitions of saturation, as they apply to their study system.

(2) Pattern, process and mechanism: stability, equilibrium and niche limitation

A major limitation to our understanding of saturation is the challenge of differentiating between the patterns resulting from saturation from the processes and mechanisms that cause it. Thus, applying a consistent terminology to each of these levels is crucial for clarity of communication and we suggest the following differentiation.

Stability is a condition where the number of species does not show a significant change in relation to a period of time or in relation to an increasing species pool. Stability represents a pattern of no directional change (but see Section II.3) and it is the first indicator that saturation might occur (Terborgh & Faaborg, 1980; Cornell & Lawton, 1992; Srivastava, 1999; Loreau, 2000; Stohlgren *et al.*, 2008*a*; Harmon & Harrison, 2015; Rabosky & Hurlbert, 2015). However, by itself stability is not sufficient to demonstrate saturation because species numbers can remain stable simply because there is no immigration or extinction in a study system (Section II.3). Hence, stability is only a pattern and we need to address the underlying processes and mechanisms to be able to document saturation.

Equilibrium in the context of saturation refers to the dynamic process whereby there is a balance of species additions (*via* immigration or speciation) and losses (*via* emigration or extinction) in a given system. As a result of this dynamic equilibrium, we observe stability in the number of species. Stability and equilibrium constitute indicators that saturation might occur. However, it is only possible to determine if saturation occurs when in addition there is also an indication that stability or equilibrium are reached by means of niche limitation and not through stochastic mechanisms.

Niche-limitation mechanisms originate from a wide range of ecological and evolutionary reasons, including, e.g. minimum population sizes, limits to the similarity of species, species interactions, and niche characteristics set by the environment. Accordingly, a full assessment of saturation requires an assessment of some of these mechanisms.

(3) How stable is stability?

That the number of species in an assemblage or lineage remains stable over time or in space provides an indication that the assemblage or lineage has reached its ecologically determined upper limit of coexisting species. However, no ecological system is totally invariable in time and space (Simberloff, 1974; Chisholm *et al.*, 2014). For example, Simberloff & Wilson (1970) interpreted their data as representing a pattern of stability (despite not using this term), but their results also show some degree of variation (Fig. 1). For example, in Fig. 1 we observe that the pre-defaunation level for island E2 was 35 species, and that following the initial recovery phase after defaunation, there was a period when species richness values varied between 30 and 40. In the context of saturation, stability then implies that species additions and losses may not necessarily be simultaneous, thus leading to a certain fluctuation of species numbers around the equilibrium level. The challenge then is defining the saturation level. Is it at the maximum (40 species), in which case the points in time with 30 – 39 species would indicate undersaturated assemblages? Or would it lie at the mean value (35 species) in which case those with 36 – 40 species would be oversaturated? Or would the saturation level be defined better as a range between 30 and 40 species, in which case the assemblage would be considered to be

continuously saturated? And in the latter case, would we still consider the assemblage to be continuously saturated if its richness fluctuates between 20 and 40 species, or even 10 and 40 species?

The complexity of the study systems, and the vast amount of potential predictors, make it hard to distinguish if these fluctuations are changes in degrees of saturation, random variations (Ricklefs, 1987; Cornell & Lawton, 1992; Cornell, 1999; Loreau, 2000), or responses to fluctuations among the ecological factors determining the upper limit of species that can co-exist. From an ecological viewpoint, these fluctuations occur as part of the colonization process. For example, oversaturation might occur when a habitat is colonized within a short time span by a large number of species and it takes some time before competitive exclusion reduces the number of species to its saturation level (MacArthur & Wilson, 1967; Heatwole & Levins, 1972; MacArthur, 1972; Gilbert & Levine, 2013). In turn, local extinction may drive species numbers below saturation levels (MacArthur & Wilson, 1967; MacArthur, 1972). Saturation thus cannot simply be defined as the maximum number of species found over the time of a study.

A comparable challenge arises when examining saturation at evolutionary timescales (Harmon & Harrison, 2015; Rabosky & Hurlbert, 2015). Is saturation only accepted if the rate of diversification (i.e. origination minus extinction) through time reaches a slope of zero, or can we already speak of saturation when the slope of the relationship decreases? In this regard, Cornell (2013, p. 158) argued that there might be a need for a new hypothesis, the ''damped increase hypothesis'', which would predict that ''diversity generally increases through time but that its rate of increase may sometimes be slowed in a diversity-dependent fashion reflective of ecological constraints''. However, it is left open whether this damped increase would eventually lead to a saturation level or continue indefinitely.

Finally, given the dynamic nature of species assemblages (Simberloff, 1974), a concept that might be useful for the assessment of saturation is that of multistability, i.e. the possibility that a system has multiple stable states as the result of its non-linear dynamics (Feudel, 2008). Under this scenario, the question of saturation as an absolute upper limit might become less relevant and turn instead towards the assessment of these 'stable' states.

We conclude that it is crucial for authors to define *a priori* how they expect their study system to fluctuate under stable conditions, which sets the stage for assessing saturation.

(4) How to assess equilibrium?

A dynamic equilibrium occurs when species additions are compensated by losses. This is a fundamental characteristic of a saturated system. Thus, one of the essential conditions to demonstrate equilibrium is that the number of species remains stable despite its potential to increase. This is important, because if for some reason no new species were to arrive or emerge in an assemblage, then the number of species would remain stable (assuming no extinction), and this could then erroneously be taken as an indication of equilibrium or even saturation. Indeed, species assemblages or lineages might reach a state of equilibrium without compensation of additions and losses (Alroy, 2008).

In addition, as already discussed above for stability, the balance between gains and losses is time-dependent and it may take decades to millennia for equilibrium states to become established (Diamond, 1972; Cornell & Lawton, 1992; Loreau & Mouquet, 1999). For example, invasive species might initially increase the species number of an assemblage (Stohlgren *et al.*, 2008*a*), but this does not automatically imply that the original assemblages were not saturated, because it might take centuries before competitive exclusion reduces species numbers to their original level (Gilbert & Levine, 2013).

In addition, Simberloff (1974) reviewed the concepts and evidence for different equilibrium scenarios. He reflected on different kinds of equilibrium (e.g. taxon cycle and dynamic equilibrium) which differ in the timescales at which they manifest and in their influence on the rates of immigration and extinction over ecological and evolutionary timescales. In this same review, the author discussed the concept of relaxation time developed by Diamond (1972), which is the time required for a system to reach equilibrium after the immigration rates have been altered by means of geological or environmental changes or by species introductions.

(5) Are there degrees of saturation?

As previously discussed, based on the example in Fig. 1 and assuming it as an indication of saturation in those islands, we observe that even saturated assemblages show certain levels of variability (which may be stochastic or ecologically driven), but it is striking that in the ecological literature, stability is generally dealt with as a binary state: an assemblage is either stable or it is not (Cornell, 1999; Srivastava, 1999; Loreau, 2000; Stohlgren *et al.*, 2008*a*). This contrasts with the literature on evolutionary processes, where reaching stability is often considered as a gradual process (Rabosky & Lovette, 2008; Rabosky & Glor, 2010; Etienne *et al.*, 2011; Gascuel *et al.*, 2015). For example, Cornell (2013) argued that diversity may already be considered to approach its upper limit when the rate of diversification decreases. He proposed the term 'damped increase' to account for such a situation. However, it is not clear whether Cornell hypothesized a damped increase with no upper limit, in which case we could not interpret slowdowns as evidence for a limit, but further diversification studies could elucidate this possibility.

Taking this line of thought a step further, one could express the degree of saturation in relation to the species richness value at which an assemblage is saturated (if this is known). For instance, in the example of Fig. 1, if one were to define saturation to be at 35 species, then at a point in time when this assemblage only has 32 species, it could be considered to be 91% saturated (or 9% under-saturated), and with 39 species, it would be 110% saturated (or 10% over-saturated). Of course, the major challenge here remains how to define the saturation limit. Partly, this is a conceptual issue, but

partly it is also a practical issue when the assemblages under consideration are not saturated and hence it is not possible to define such a limit empirically. However, even if the saturation level is unknown, certain patterns such as a deceleration of species immigration may be taken as an indication that an assemblage or a lineage is approaching saturation. Thus, it is more saturated (or less under-saturated, as one prefers) when the immigration rate is low than when it is high. The same argument is valid for saturation at the evolutionary timescale, although obviously at vastly different timescales and with different underlying processes and mechanisms.

Thus, a further conclusion is that it may be more meaningful to consider saturation not as a binary concept but rather as a ratio.

(6) The importance of the referential system

Saturation in chemistry is defined in relation to certain conditions of temperature and pressure (Encyclopaedia Britannica, 2011). Accordingly, in ecology and evolution saturation defined as the maximum number of species that can coexist depends on where, when, and of whom. Because biological systems change over time, a system can or cannot be saturated or can reach a different degree of saturation according to the area and energy available for the organisms, and the evolutionary dynamics of the taxonomic group (Ricklefs, 1987; Cornell & Lawton, 1992; Lawton, 1999; Fine & Ree, 2006; Cornell, 2013).

Once again, we can take Fig. 1 as an indication of saturation in that species richness remained stable over time and rebounded after a major disturbance. However, this does not imply that this is the maximum upper limit of insect species that can co-occur on these islands over evolutionary timescales. What if a completely new insect group were to evolve and use currently unexploited niche space on these islands (as e.g. Fordyce, 2010)? It is likely that overall species richness would increase, just as, e.g. local mammal diversity increased after the exploitation of the aerial niche space following the evolution of bats (Wilson & Reeder, 2005; Simmons *et al.*, 2008), marine fauna diversified after the massive extinction events of the early Ordovician and late Permian (Fig. 2; Raup & Sepkoski, 1982; Sepkoski, 1984; Benton, 1995; Brayard *et al.,* 2009), and feathered dinosaurs ‘‘evaded the effects of niche saturation’’ and prompted the radiation of birds (Benson *et al.*, 2014). Alternatively, and returning to the hypothetical case of the insect fauna, let us suppose that a major global extinction event decreases world-wide insect richness below today’s levels. A local defaunation experiment might then still find evidence for saturation in that species richness would reach stable pre-defaunation levels after some time. Still, this level would be lower than today’s saturation level. Based on our current knowledge, we would know that the island habitats have the capability to support more insect species or have a different level of saturation under different circumstances.

The issue here is that the maximum number of species that can co-occur in an assemblage at a certain point in time is not only determined by the habitat conditions (the carrying capacity), but also by the number of species available to colonize the habitat (Cornell, 1999; Srivastava, 1999; Alroy, 2008; Cornell & Harrison, 2014). This regional species pool, from which a local assemblage results, varies in time and space, and accordingly, so will the level of saturation (Terborgh & Faaborg, 1980; Srivastava, 1999; Cornell & Harrison, 2014; Karger *et al.*, 2015).

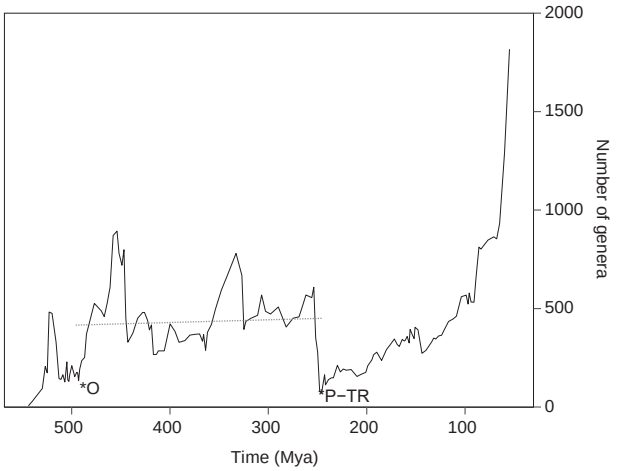

Fig. 2. Diversity of marine animal genera through the Phanerozoic (modified from Sepkoski, 1984). Two major extinction events are indicated by asterisks. Note that after the Ordovician extinction (*O), a strong initial increase occurs and that then the number of genera stabilized for almost 250 million years, suggesting saturation (dashed grey line, $r^2 = -0.01$, $F = 0.24$, $P = 0.62$). However, after the Permian–Triassic extinction event (*P–TR), diversity increased beyond the original saturation level ($r^2 = 0.53$, $F = 77.35$, $P = 1.0e^{-12}$) possibly as a result of major evolutionary innovations that opened new niche space. Mya, million years ago.

Furthermore, saturation of a given assemblage may be assessed both in space and in time. This may be exemplified by contrasting two classical studies, that of Elton (1950) and that of Simberloff & Wilson (1969). Elton (1950) examined animal species richness by comparing several assemblages at the same point in time, finding that in similar habitats, species richness was roughly constant, and interpreting this as an indication of saturation. By contrast, Simberloff & Wilson (1969) studied the temporal change in species richness for a single assemblage. What is crucial to consider here is that the same assemblage may be assessed as being saturated under one referential system but not under another.

As an example, we could consider three approaches to studying the biota of an island, which after defaunation is in the process of being recolonized and still has increasing species richness. If we study this island in (*i*) a temporal framework following Simberloff & Wilson’s (1969) approach, we will find that species richness is still increasing, and conclude that the assemblages are unsaturated. However, if (*ii*) we compare the assemblages at different sites within the island, following Elton’s (1950) approach, we might find similar richness in comparable habitats and deduce that the

assemblages may be saturated (pending evidence for niche limitation). Yet, if (*iii*) we compare the island assemblages to those on the mainland, we would find lower richness on the island and conclude that the assemblages are unsaturated. Note that in these examples the difference is not only one of time *versus* space, but also of our implicit definition of the species pool. In examples (*i*) and (*iii*), our species pool is the regional flora and fauna from which the species that colonize the island are drawn. In example (*ii*), we implicitly restrict the species pool to include those species present on the island at the time of the study. So, are these assemblages saturated or not? We consider that this question cannot be answered in absolute terms, but only with reference to the framework within which we assess saturation. Which framework is appropriate will depend on the biological question being asked. If our question is about whether the island is capable of supporting more species, then approaches (*i*) and (*iii*) would be appropriate. But if we wanted to know if the species already present on the island are using the available resources to the maximum that is possible with the present biota, then approach (*ii*) would be appropriate.

So far, we have discussed the aspects of the referential system related to the spatial and temporal coordinates; these aspects refer to the questions of saturation where and when. A third important aspect is that of saturation of whom. Saturation can be studied in groups that share a common ancestor (e.g. a taxon or a lineage) or those that do not (e.g. an assemblage or guild), but the fundamental aspect to be considered is if the targeted species potentially interact or not. This is important because it is the base for niche-limitation mechanisms and sets the stage to defining the appropriate space and timescales and the respective species pool (Section III.3), and in the case of equilibrium, if saturation will be defined in terms of immigration/emigration (Section III.4) or in terms of speciation/extinction rates (Section III.5).

We highlight that careful consideration needs to be given to defining the appropriate referential system for the questions at hand. This applies to all methodological approaches outlined in Section III.

(7) What to measure: diversity, species or traits?

A factor that may also affect the conclusions drawn about saturation is whether the diversity of the studied assemblages is evaluated only based on species richness or whether some index of diversity is used which weights species based on their abundances (Magurran & McGill, 2011). Both approaches have their justification based on the specific questions asked, but it must be clearly stated which of them is used and why. As an example, Fig. 3 shows a data set for fish species recorded over 25 years in the Bristol Channel, and analysed using two different methodological approaches. The first study focused on raw species richness, where a marked increase was found (Henderson, 2007). The second study used a diversity index instead of raw data resulting in a less steep relationship (Magurran & McGill, 2011). In the context of the stability debate, these two approaches may thus lead to different conclusions.

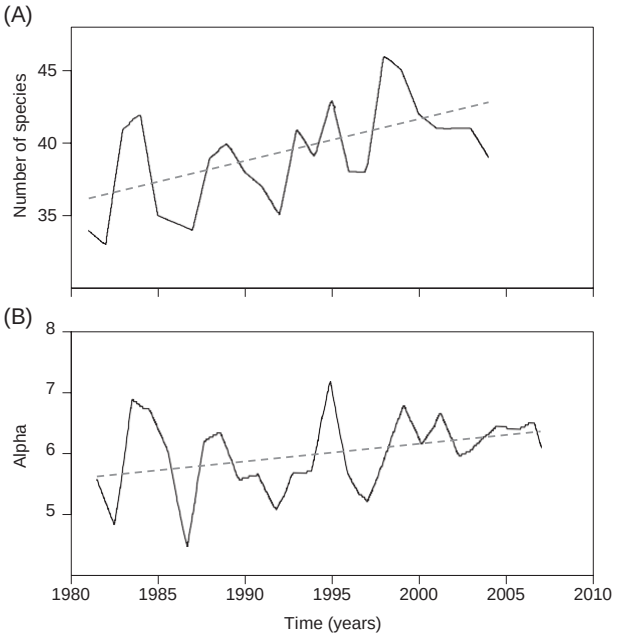

Fig. 3. Fish species richness through time in the Bristol Channel Estuary, UK. (A) Using the number of species observed against time (modified from Henderson, 2007), an increasing trend is detected (r^2 0.39, F 1347, $P < 2.2e^{-16}$). (B) Fisher's alpha values for the same data (modified from Magurran & McGill, 2011) show a less pronounced relationship ($r^2 = 0.14$, $F = 158.8$, $P < 2.2e^{-16}$).

Another variant is the use of functional richness instead or in addition to taxonomic richness. Functional richness refers to the niche space filled by species in an assemblage (Mason *et al.*, 2005). A functional group is defined as a group of species that share traits linked to ecological functions, in particular those related to the use and competition for resources or to physiological constraints imposed by the environment (Kraft, Godoy & Levine, 2015; Díaz *et al.*, 2016). The idea of assessing functional saturation is to evaluate whether an increase in the number of 'taxonomic entities' translates into an increase in 'ecological entities'. For instance, Canning-Clode *et al.* (2010) used this approach experimentally to evaluate the local– regional richness relationship in artificial marine assemblages. They set bare vinyl panels in analogous marine habitats in different regions of the world and monitored their colonization and succession over 2-month periods for a total of 8 months. Species were classified into functional groups according to traits related to body size, growth form, modularity, motility, and trophic type. The local *versus* regional richness regression analyses demonstrated that for both functional and species richness, assemblages seemed to be saturated at initial and late successional stages. At intermediate stages, however, both types increased with their respective regional richness, indicating under-saturation.

A difficulty in assessing functional saturation is a lack of knowledge on functional biology for most groups of animals and plants; the use of morphological niche volume might

be a way to approach this problem. A morphological niche volume is a 'space' where all key morphological traits of the group of interest occur (Díaz *et al.*, 2016). Hence, saturation would be assessed by comparing the portion of the volume occupied by local assemblages.

In evolutionary terms, we can also think about the evolutionary potential of a specific clade as a reference system, i.e. the potentially limited number of morphological trait combinations that a clade can attain. Using this reference, we can then think of testing saturation of the 'potential morphological volume'. If all of the possible combinations have evolved, this would be indicative of saturation.

III. METHODS TO ASSESS SATURATION

We now summarize methodological approaches for assessing saturation identified in our literature survey. Three general groups were recognized according to the patterns, processes, and mechanisms that define saturation: stability, equilibrium, and niche limitation. We subdivided these groups into subcategories in relation to the temporal timescale, and when needed to the specific aspect assessed by each method.

(1) Stability in ecological time

(a) Species accumulation

(*i*) *Approach.* This is the approach of Simberloff & Wilson (1969, 1970) in which species numbers are tracked over time after a natural or anthropogenic event has caused an obvious reduction in species numbers (Fig. 1). To be taken as an indication of saturation, it must be shown that (*i*) the number of species stabilizes, or (*ii*) reaches the number of species present before the reduction event (if known).

This approach has been used mainly for life forms or ecological guilds, irrespective of their phylogenetic relationships (e.g. Whittaker, 1977). The rationale behind this is the assumption that there is ecological interaction among the species of the selected group.

(*ii*) *Examples.* Examples of this approach are studies of colonization of the Krakatau Islands (Fig. 4). Periodical plant surveys on the different islands showed that after the volcanic eruption of 1883, there was a continuous increase of species numbers on both the larger island of Rakata and the smaller island of Sertung, although the rate decreased after 1910 (Fig. 4). A decline on Sertung, particularly strong between 1920 and 1940, suggests that its plant assemblages are more saturated than those on Rakata.

(*iii*) *Evaluation.* Two of the challenges outlined in Section II are particularly relevant here. First, consideration on how to deal with temporal variability is needed (Section II.3). Second, a definition is needed on whether a partial reduction in the accumulation curve is accepted as indicative of saturation or if only a slope of zero is accepted (Section II.5).

Several studies have used species accumulation curves (SACs) over time (Yosef & Tryjanowski, 2002; de de Souza, Marinoni & Marinoni, 2014; Kesting, Petersen & Isselstein, 2015) or in combination with productivity (Guo, Shaffer & Buhl, 2006; Ptacnik *et al.*, 2010; Di Pippo *et al.*, 2014) as tests for saturation. While this may be appropriate in specific cases, we call attention to the risk of confusing sampling completeness with saturation. Assessments of saturation must be based on either complete or equally comprehensive sampling, and to show that the number of species recorded at a site stabilizes over time or with productivity is in no way an indication of saturation at this site.

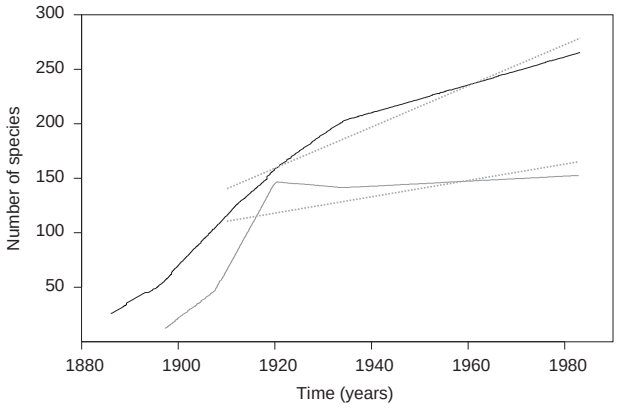

Fig. 4. Number of vascular plant species through time on two of the oldest islands in Krakatau (modified after Whittaker, Bush & Richards, 1989). On Rakata (black solid line), the species number increased continuously despite an accumulation decline after 1910 (top dashed line, r^2=0.94, F =6368, P < 2.2e^{-16}). On Sertung (grey solid line) the number of species increased steadily until about 1920, a subsequent decline followed until about 1940, then it continued increasing but at a lower rate (bottom dashed line, r^2= 0.52, F =658.8, P < 2.2e^{-16}). Results on both islands indicate undersaturation, particularly on Rakata.

(b) Number of species

(*i*) *Approach.* This is comparable to the previous approach, with the difference that there is no initial reduction in species numbers, so that all that is assessed is whether species numbers show a trend in time.

(*ii*) *Examples.* Long-term monitoring of natural assemblages constitutes the most common example of this kind of assessment of saturation. Figure 5 shows that the number of bird species recorded from Eastern Wood, UK, remained within the range 27 – 35 species over a 30-year period (Gaston & Blackburn, 2000). Importantly, the total number of species recorded over the years increased over time, showing the ability of additional species to colonize the study region (Section II.3).

(*iii*) *Evaluation.* One of the important challenges when using this approach is variability (Section II.3). The length of the time record is also important and should consider the natural variation in species numbers because short sampling periods might only show these fluctuations, but in the long term they might be part of a stable trend.

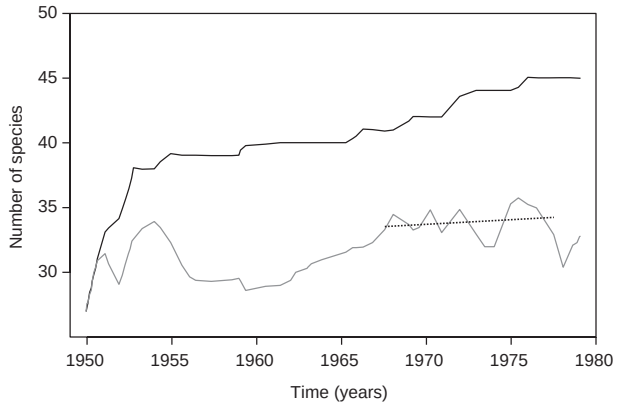

Fig. 5. Number of bird species recorded in Eastern Wood, UK, between 1949 and 1979 (modified after Gaston & Blackburn, 2000). Overall, the number of species per year (grey line) has been increasing over time ($r^2 = 0.34$, $F = 36.46$, $P = 7.60e^{-08}$), albeit with some periods of decline and stability, e.g. between 1967 and 1977 (black dashed line; $r^2 = 0.03$, $F = 0.53$, $P = 0.47$). New species were able to reach the assemblage, increasing the cumulative number of species over time (black line; $r^2 = 0.79$ $F = 257.2$, $P < 2.2e^{-16}$). This is indicative of local saturation coupled with species immigrations and extinctions.

(c) Species introductions

(*i*) *Approach.* This approach is based on the study of stability of species richness in ecological time, but with the purposeful or unplanned 'experiment' of species introductions. The basic assumption is that if assemblages are saturated, then after species introductions they should return to the original level of species richness. Methods included under this approach might be similar to those used in Sections III.1*b*, III.3 or a combination of both.

(*ii*) *Examples.* The first example is a study of vascular plants in Northwest and Central USA, where Stohlgren *et al.* (2008*a*) assessed local species richness in plots of 100 m^2 and related it to regional richness. Regional richness was defined as the sum of species richness found in five 1000 m^2 plots of the same vegetation type. The authors argued that richer local sites should show an asymptotic relation relative to the species pool. They found however a linear relationship between local– regional richness for each vegetation type, and argued against a saturation signal in these assemblages.

In another example, Akatov & Akatova (2010) studied the proportion of native and invasive plant species in open coastal shores of mountain rivers in the western Caucasus. Saturation was assessed by comparing the composition and structure of plant assemblages in plots. The authors assumed that plots located in areas of higher richness should have a higher level of saturation than plots of the same area in poorer assemblages. By doing so, the authors pre-established the level of saturation and then assessed the assemblages 'resistance' to invasive species.

(*iii*) *Evaluation.* Using species introductions to assess saturation is, in principle, a suitable approach but it is riddled with challenges. First, it is known that for a while following introductions, assemblages may be oversaturated (Sax *et al.*, 2007) and that it may take decades or longer before species numbers stabilize (Gilbert & Levine, 2013). The challenge here is determining whether an assemblage is still in this lag phase or has indeed stabilized at a higher level of species richness.

Second, in the examples above, the authors compared assemblages of originally different species richness. Clearly, there must be biological reasons for these differences, e.g. related to climate or nutrient availability. It is thus hard, if not impossible, to predict the susceptibility of these different assemblages to introduced species (Stachowicz & Tilman, 2004; Fridley *et al.*, 2007).

Third, invasive species may include novel physiological or ecological traits or adaptations not present in the original assemblage (Sax *et al.*, 2007). In such a situation, the original assemblages may have been saturated relative to the original species pools, but not in absolute terms. Here, the issue of the referential system becomes relevant (Section II.6).

Fourth, there is the problem of spatial scale. Numerous studies have shown that locally, invasive species can reduce local species numbers, e.g. when an invasive aquatic species excludes other species (Canonico *et al.*, 2005). However, this rarely leads to total regional extinction of the original species (Gurevitch & Padilla, 2004; Fridley *et al.*, 2007, but see Clavero & García-Berthou, 2005). Thus, at the regional scale, the biota may not be considered saturated, whereas at the local scale it would be.

More generally, if positive effects of introductions on species richness are interpreted as indicative of undersaturation, how do we interpret negative effects of introduced species? If, for example, an invasive species of *Acacia* covers hundreds of km^2 of fynbos habitat in South Africa and leads to the extirpation of dozens of native species (Richardson, Macdonald & Forsyth, 1989), resulting in both local and regional reductions of species numbers, does this imply that the original assemblages were oversaturated? Obviously not. Clearly, much care should be taken to interpret such cases in terms of the saturation discussion.

(2) Stability in evolutionary time

(a) Species accumulation

(*i*) *Approach.* The basic approach is equivalent to that in Section III.1*a*, with the difference residing in the temporal and (usually) spatial scales that are covered. Here the number of species is tracked over evolutionary time after an event has caused a significant reduction in species numbers. There are two fundamentally different data sources for this approach: phylogenetic reconstructions derived from molecular analyses of usually extant taxa, and fossil records.

(*ii*) *Examples.* Basically, any dated phylogeny exemplifies this approach because it documents changes in the number of species of a lineage(s) through time. Figure 6 shows the number of species through time for three different groups using the model developed by Etienne *et al.* (2011). The three groups show a slowdown of diversification

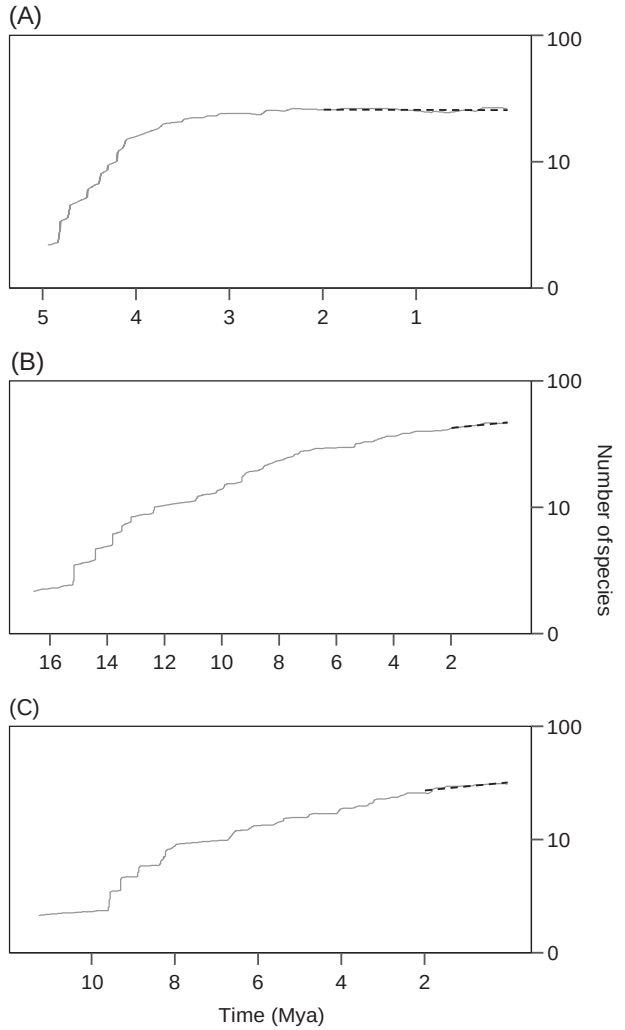

Fig. 6. Number of species through time as predicted only from molecular phylogenies [modified after Etienne *et al.*, 2011, original data from Rabosky & Lovette, 2008 for *Dendroica* (A), Fordyce, 2010 for *Heliconius* (B), and Kozak, Weisrock & Larson, 2006 for *Plethodon* (C)]. Linear models suggest saturation for the warbler genus *Dendroica* which had only a slight increase in the number of species during the last 2 million years (Mya) ($r^2 = 0.016$, $F = 4.434$, $P = 0.04$). The butterfly genus *Heliconius* and the salamander genus *Plethodon* show an ongoing increasing trend during the same period ($r^2 = 0.84$, $F = 308.4$, $P < 2.2e^{-16}$; $r^2 = 0.798$, $F = 357.4$, $P < 2.2e^{-16}$, respectively).

rates, but only North American wood warblers have reached a plateau consistent with saturation (Fig. 6A). Many other studies illustrate this pattern, e.g. on the diversification patterns of marine invertebrates (Rabosky & Hurlbert, 2015), ammonoids after the late Permian mass extinction (Brayard *et al.*, 2009), mammals, in particular for the families Bovidae, Herpestidae, Ziphiidae, Ochotonidae and Talpidae (Soria-Carrasco & Castresana, 2011), and the plant genus *Tetraria* (Cyperaceae) from the Cape fynbos (Slingsby, Britton & Verboom, 2014).

(*iii*) *Evaluation.* When using phylogenetic methods, one of the major challenges is to estimate the past number of species given that extinction plays a determinant role (Dera *et al.*, 2010; Marshall, 2017). A classic example is that of the diversification of cetaceans, where a phylogeny based only on the extant taxa indicates that species numbers have been increasing, whereas inclusion of the fossil record shows that species numbers were much higher in the past and have been decreasing since the Miocene (Etienne *et al.*, 2011). Thus, while methods with and without fossils are suitable as tests of saturation, by using only the latter, one should be aware that while signs of slowing down or 'damped' diversification might be observed (as e.g. in Harvey, May & Nee, 1994), the asymptotic pattern is less likely to be observed.

(b) *Number of species*

(*i*) *Approach.* Stability here implies that the number of species is constant over geological time periods.

(*ii*) *Examples.* A first example of this approach was presented in Fig. 2, where the marine fauna record by Sepkoski (1984) suggests a saturated number of genera between the early Ordovician and late Permian. Another example is a study of ammonoid richness after the late Permian extinction event (McGowan, 2005). Figure 7 illustrates the percentage of ammonoid genera in different periods of the early Triassic. Results show that after an initial increase, the percentage of ammonoid genera reached stability. Finally, a fossil record study suggests that several wetland plant assemblages reached a state of stability during the Carboniferous (Cleal *et al.*, 2012).

(*iii*) *Evaluation.* Large variation in the number of species over geological time is one of the arguments against saturation (Harmon & Harrison, 2015). Hence, again the length of the time record is of major importance in the detection of stability trends, because as Rabosky (2009) explained, the observed trend might vary according to the evolutionary stage of the study group. If only the initial 'growth' phase of clades is captured, then a continuous increasing trend will be observed, but this trend might show a decline and further stability in later stages when the growth phase has receded.

(c) *Species introductions*

(*i*) *Approach.* This approach is analogous to that on species introductions in ecological time (Section III.1*c*), but at different spatial and temporal scales, with species 'introductions' usually the result of long-distance dispersal between continents or biogeographical regions.

(*ii*) *Examples.* Pinto-Sanchez *et al.* (2014) aimed to find evidence of saturation by studying the impact of the Great American Biotic Interchange (GABI) on patterns of local richness of a group of frogs. They traced the Central or South American origin of each lineage and identified those that dispersed among regions during the GABI. By estimating local species richness in each region before and after the GABI, they assessed the local changes in both invaded assemblages. Since local species richness increased

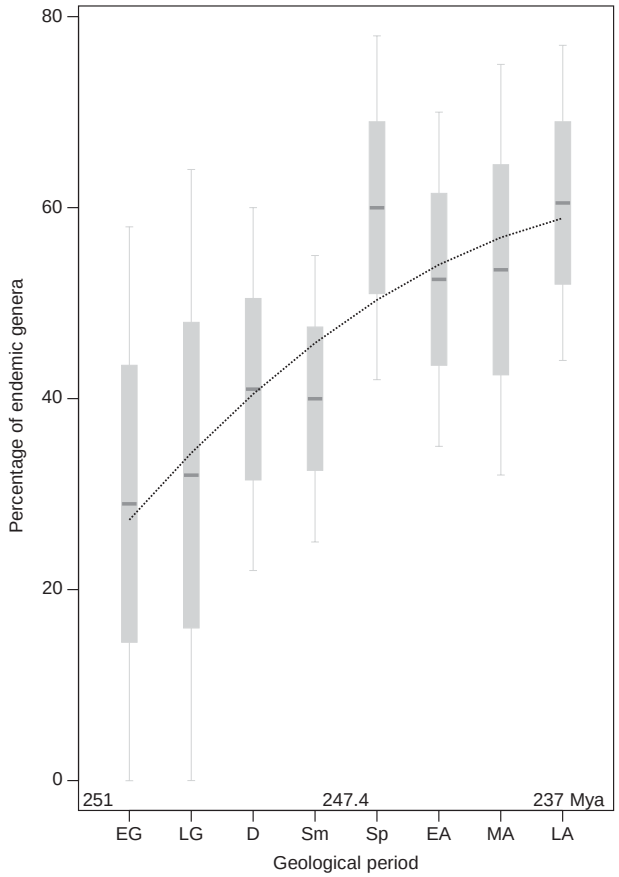

Fig. 7. Patterns in ammonoid endemic genera during the early Triassic (modified after McGowan, 2005). The boxplots show the range (boxes and whiskers) and median (midlines) values of the percentage of endemic ammonoids in each geological period. These values describe an asymptotic curve (r^2 = 0.37, F = 102.9, $P < 2.2e^{-16}$) with no significant change during the last four periods (r^2 = 0.006, F = 0.01, P = 0.92). EG = Early Griesbachian; LG = Late Griesbachian; D = Dienerian; Sm = Smithian; Sp = Spathian; EA = Early Anisian; MA = Middle Anisian; LA = Late Anisian. Mya, million years ago.

in both assemblages, they concluded that assemblages must have been unsaturated before the GABI.

(*iii*) *Evaluation.* The challenges of this approach are analogous to those outlined at the ecological timescale (Section III.1*c*).

(3) Stability relative to the regional species pool

(a) *Approach*

Under this approach, saturation occurs when the relationship between the local *versus* regional species pool reaches an asymptote (Terborgh & Faaborg, 1980).

(b) *Examples*

The study of Terborgh & Faaborg (1980) aiming to test saturation of bird assemblages in the West Indies is an early application of this approach. The authors defined the total number of species present on each island as the insular species pool and then evaluated the local species richness at sites in different habitats on each island. The number of bird species in each habitat increased linearly until it reached a limit, which was interpreted as indicative of saturation on the large islands (Fig. 8).

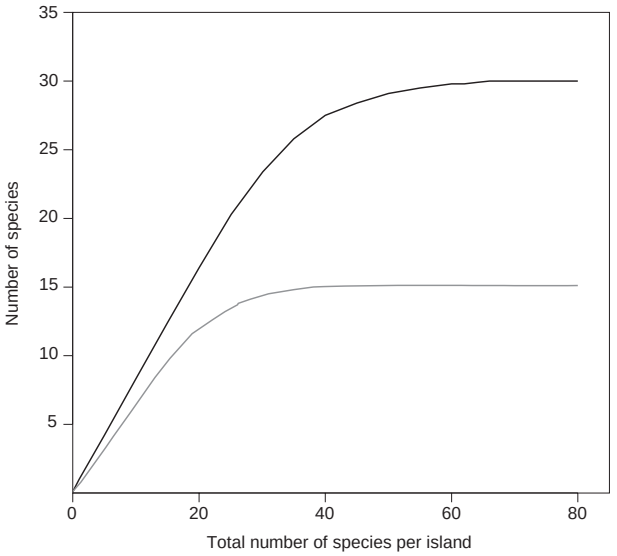

Fig. 8. Number of bird species observed in two habitats (sclerophyll scrub, black; rainforest, grey) against the total number of species on 12 Greater and Lesser Antillean islands (modified after Terborgh & Faaborg, 1980). In this study, the relationship between the total number of bird species on each island and the number of species observed in each habitat had two phases. In an initial linear phase, the number of species in each habitat increased as more species were present in the island; in a second phase the number of species per habitat remained constant despite an increased number of species on the island (scrub: $t = 1.47$, $P = 0.17$; rainforest: $t = 1.64$, $P = 0.11$). The latter phase was interpreted as indicative of saturation.

More recent examples include a survey of local and regional species richness of Collembola in ten grassland types (Winkler & Kampichler, 2000). The regression analyses of local *versus* regional species richness demonstrated a pervasive pattern of stability across all grassland types. In a further study, Patzkowsky & Holland (2003) studied the relationship between local and regional diversity in tropical marine assemblages during 13 million years (Mya) of the Late Ordovician. They concluded that local assemblages did not show stable species numbers but instead that local richness was determined by changes in the regional species pool caused by oceanographic processes. Finally, Krasnov *et al.* (2006) analysed records of flea diversity on 28 small mammal species. In this study, the species pool was defined as all flea species present on a host species, i.e. each host species counted as an independent 'region'. Stability was supported by the asymptote found for all flea assemblages studied.

(c) Evaluation

Despite its apparent simplicity, the local–regional approach to test for saturation is also riddled with challenges (Srivastava, 1999). The first issue deals with adequately defining the scale of the local sampling units (Fox *et al.*, 2000; Shurin *et al.*, 2000). If the local units are so small that there is a limit to the number of individuals that approximates the potential number of co-occurring species, then local richness remains stable due to a sampling effect (Jones, Tuomisto & Olivas, 2008). Take the simple example of a forest assemblage in which local richness is sampled in plots that allow the presence of 100 tree individuals. This size may be adequate if the regional species pool is limited to a few dozen species. However, if there are thousands of tree species in the area, it will be impossible to record more than 100 species in a plot, irrespective of changes in regional species numbers. It is thus crucial to test whether local richness has been sampled in a representative manner, e.g. by using species-richness estimators that estimate the actual number of species in a sample based on subsamples (Gotelli & Colwell, 2001). Only if sampling completeness is equivalent across local sampling units can stability be tested for (Shurin *et al.*, 2000).

The appropriate definition of the regional species pool is even more complex. The local–regional test for saturation is based on the idea that the regional pool includes all species that could potentially occur within our local sampling units at a given period in time (Cornell & Harrison, 2014; Kraft & Ackerly, 2014). The challenge of defining the regional pool becomes evident especially in a mainland situation, where simply using the total number of species recorded at a certain distance around a local sampling unit (regional richness) is likely to include species that will not be able to colonize the local site while at the same time excluding species from further afield that may well make it into our local site (Pärtel *et al.*, 1996; Lessard *et al.*, 2012; Cornell & Harrison, 2014). It is in such a situation where analytical (Eriksson, 1993; Dupre, 2000; Carstensen *et al.*, 2013) and probabilistic species pool approaches may be appropriate (Ewald, 2002; Fukami, 2015; Karger *et al.*, 2016). This does not simply include or exclude a species in the pool based on subjective criteria but assigns a certain probability of a species belonging to the species pool, based on the ecological requirements, distribution, and dispersal ability of the species (Zobel, van der Maarel & Dupré, 1998; Karger *et al.*, 2016). The approach also allows one to incorporate the influence of regional (e.g. dispersal) and local factors (e.g. habitat availability) on local assemblages (Hillebrand, 2005; Harrison & Cornell, 2008).

(4) Equilibrium in ecological time

(a) Approach

This tests the hypothesis that given enough time and under stable environmental conditions, if saturation is present, then the rates of immigration and extinction of a study group balance each other within an area where the species interact.

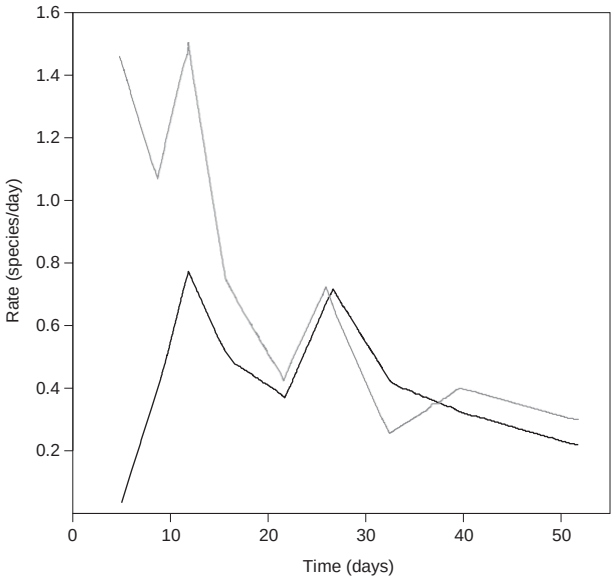

Fig. 9. Average immigration (grey line) and extinction (black line) rates of ciliate species in 42 experimental islands (modified from Have, 1987). After day 20 the rates converged to similar trajectories without significant differences ($t = -0.51$, $P = 0.61$).

(b) Examples

Originally, Simberloff & Wilson (1969, 1970) tried to test this hypothesis in their defaunation experiment, in which they removed all insects from small mangrove islands in Florida, USA, and recorded their recolonization by frequent censuses during a 2-year period. The authors hypothesized that as species richness values rebounded to those before removal, the rates of immigration and extinction should converge towards similar values. However, the observed and simulated curves differed too much from each other and also within themselves, precluding the authors from reaching any conclusion.

Have (1987) created a microcosm experiment to demonstrate the convergence of immigration and extinction in ciliate assemblages. In this study, 42 cylinders of different size were mounted on a platform and immersed in a freshwater pond in Denmark to simulate island habitats for ciliate species. The presence and absence of morphospecies was recorded over time (Fig. 9). As predicted by the island biogeography theory, the rates of immigration and extinction converged after 20 days.

A further example is the equilibrium of bird species in the Channel Islands (Diamond, 1969, 1970). Here, most of the islands were found to be in equilibrium because between 17 and 62% of the resident (breeding) species in 1917 disappeared and were replaced by a similar number by 1968. Finally, Crowell (1973) introduced and monitored populations of different mouse species in small islands

of Penobscot Bay, Maine, USA from 1962 to 1972. He concluded that mice were able to establish and reached equilibrium through a balance of immigration and extinction during this period.

(c) Evaluation

Two challenges are of major importance when using this approach. The first is variability in the rates of immigration and extinction, which precluded Simberloff & Wilson (1969, 1970) from demonstrating their convergence. Simberloff (1969) also attempted to demonstrate it by obtaining simulated curves, but without success. The second challenge is the time needed to obtain a data series that captures the trends of immigration and extinction. Using short-living organisms is one way to overcome this challenge (Have, 1987; Fox *et al.*, 2000; Soares, Schoereder & De Souza, 2001), but for many others, long-term experiments and monitoring is the only solution.

(5) Equilibrium in evolutionary time

(a) Approach

Here equilibrium is reached when the rates of origination and extinction balance each other. In practice, this implies that an initial increase in the diversification rate of a lineage is compensated by a slowdown that might be caused by a decline in speciation rates or by an increase in extinction rates. As in general for evolutionary-scale studies, this can be studied using phylogenetic or fossil data.

(b) Examples

One of the best-known examples of this approach is the diversification of the Caribbean lizards in the Greater Antilles. Rabosky & Glor (2010) argued that while the number of *Anolis* lizards might still be increasing on the largest island Cuba, the number is relatively stable on Hispaniola, Jamaica and Puerto Rico. The reason for this equilibrium is the convergence of speciation and extinction rates towards similarly low values.

The diversification of the wood warbler genus *Dendroica* is another example. Both Rabosky & Lovette (2008) and Etienne *et al.* (2011) found that the number of *Dendroica* species has been stable during the last 2 Mya (Fig. 6A). Also, under a maximum likelihood linear diversity-dependence model, rates of origination and extinction have been at equilibrium since then (Etienne *et al.*, 2011). A further example of equilibrium over evolutionary time is given by Valente *et al.* (2017) for bird species on Atlantic islands.

(c) Evaluation

An important aspect to consider here is that equilibrium as an indicator of saturation is specifically defined as the balance between the rates of origination and extinction, because as explained by Alroy (2008) equilibrium in evolutionary time might occur without this condition.

(6) Niche limitation in ecological time

(a) Approach

This approach tests the hypothesis that the number of species in a given ecological time and area is constrained by a limited number of available niches.

(b) Examples

In a study of birds in the Channel Islands, Jones & Diamond (1976) demonstrated that the lack of a significant change in the total number of breeding birds between 1917 and 1968 (Diamond, 1969) was in many cases the result of competition among species.

Brown (1959) characterized the local distribution of ant assemblages in coconut plantations of the Solomon Islands, finding that different ant species with similar niche preferences would constantly fight and replace each other in the territory they occupied. These fights were more frequent in areas with fewer available niches, i.e. in areas with less diversity of vegetation and habitat heterogeneity. On the contrary, several ant species coexisted in areas with greater heterogeneity.

Levins & Heatwole (1973) also demonstrated niche limitation on a small island near Puerto Rico. Their approach consisted of introducing and monitoring different species of lizards and ants with equivalent niches to the native fauna. The introduced species were able to establish but later became extinct as a result of competition with previously resident species.

Many other studies provide either theoretical, empirical or experimental evidence of niche limitation mechanisms, e.g. studies on minimum population sizes (Cornell, 1999), limits to the similarity of species (MacArthur & Levins, 1967), species interactions (Wilbur, 1972; Shurin & Allen, 2001; Gotelli & Rohde, 2002; Stachowicz & Tilman, 2004; Northfield *et al.*, 2010), niche characteristics set by the area (Rosenzweig, 1995; Ricklefs, 2004; Linder, 2008) and environment (Wiens, 1974; Tilman, 2004; Fierer & Jackson, 2006; Starzomski *et al.*, 2008; Jetz & Fine, 2012).

(c) Evaluation

The role of niche limitation in determining community composition covers a huge literature and has been reviewed by various authors (e.g. Elton, 1946; Lawton & Hassell, 1981; Jeffries & Lawton, 1984; Tilman, 2004; Abrams & Cortez, 2015). It would be beyond the scope of this review to explore fully all issues related to this, particularly because most studies on the roles of niche limitation were not concerned with saturation. Indeed, demonstration of niche limitation by itself is not direct evidence of saturation, rather one of several prerequisites necessary to assess it. Nevertheless, one of the crucial aspects is that a profound understanding of the natural history of the interacting species is important because as illustrated in the previous examples, niche-based displacement sometimes only occurs between 'ecological homologues', or in other cases the

effect occurs through indirect competition (Bonsall & Hassell, 1997).

(7) Niche limitation in evolutionary time

(a) Approach

This is an approach rooted in the concept of niche filling (Schluter, 2000; Losos & Miles, 2002), testing the hypothesis that the number of species in a given evolutionary time and area are constrained by a limited number of niches available.

(b) Examples

For *Anolis* lizards in the Caribbean, Mahler *et al.* (2010) demonstrated that the rate of evolution of two important adaptive characters (body size and limb length) decreased in relation to the number of coexisting species on these islands. *Anolis* species on the Greater Antillean islands showed the greatest reduction in the rate of evolution of these traits as a result of a higher density of coexisting species. These results suggest that the number of *Anolis* species is constrained by the limited number of niches that are available.

Another study used the order of trait divergence in the woody plant genus *Ceanothus* to provide evidence of niche limitation (Ackerly, Schwilk & Webb, 2006). The study showed that closely related members of the genus rapidly developed divergence in leaf area, an important adaptive trait, which might in turn have allowed the local coexistence of different species with overlapping distributional ranges.

The niche pre-emption hypothesis (Silvertown, 2004) suggests that niche space is occupied sequentially, and thus taxa arriving earlier to an empty niche would have an advantage over latecomers. Testing this hypothesis, Tanentzap *et al.* (2015) demonstrated that the earlier colonization of plant genera in the alpine assemblages of New Zealand limited the diversification of other genera that arrived later to the same area.

Further studies have documented competitive exclusion based on fossil data, for instance for multituberculate mammals (van Valen & Sloan, 1966; Krause, 1986), bryozoans (Sepkoski, McKinney, & Lidgard, 2000), barnacles (Stanley & Newman, 1980), and bivalves and brachiopods (Liow, Reitan & Harnik, 2015). Importantly, many of these studies come from the marine fauna, where a comprehensive fossil record makes tracking interactions among taxa over evolutionary timescales possible.

(c) Evaluation

Currently, this approach depends heavily on morphological and functional characterization of the study group, and therefore a good knowledge of the adaptive importance of traits is required to assess saturation over evolutionary timescales (Mahler *et al.*, 2010). Still, there are numerous difficulties in demonstrating niche limitation at evolutionary timescales, for instance if the result of such limitation is a directional trait displacement and this involves not one but many morphological or functional attributes, or if the signal of such displacement is lost over time (Anacker & Strauss, 2014). Another aspect to consider is that detecting niche limitation is plausible for sympatric lineages but much less straightforward for allopatric lineages. The issue here is that for allopatric lineages, niche filling is driven by the interaction among species that are not closely related and hence phylogenetic methods might not be the most appropriate (Mahler *et al.*, 2010).

IV. SYNTHESIS

Historically, many assumptions and hypotheses related to the processes and mechanisms that underlie saturation have been mixed with the study of patterns that result from several ecological phenomena, leading to mixed and inconclusive results (Terborgh & Faaborg, 1980; Srivastava, 1999; Harrison, 2008; Stohlgren *et al.*, 2008*a*,*b*; Harmon & Harrison, 2015; Rabosky & Hurlbert, 2015). Trying to infer the mechanisms by studying the pattern or *vice versa* is fraught with challenges (see e.g., Hillebrand & Blenckner, 2002). We contend that by separating stability, equilibrium, and niche limitation we can achieve a more explicit research agenda in which the hypotheses, sampling design, and results distinguish between assessing the patterns, processes, and mechanisms of saturation, respectively. By often combining them, studies have obtained results that commonly cannot truly demonstrate saturation, nor provide clues for the mechanisms behind it (Cornell, 1999; Lawton, 1999; Loreau, 2000; Shurin & Srivastava, 2005).

To assess saturation, it is thus necessary to demonstrate: (i) stability, under the condition that other species must be able to immigrate or originate *and/or*, (*ii*) equilibrium, under the same condition than (*i*), *and* (*iii*) that such stability and/or equilibrium are the result of a niche-limitation mechanism. Conversely, saturation can be rejected as a hypothesis if the study system can be shown not to meet the criteria of either stability, equilibrium, or niche limitation.

Following these criteria and by gathering information from different sources, we can highlight two study systems in which saturation has been fully assessed, one at an ecological timescale and the other in a more evolutionary context. First, for breeding birds on the Channel Islands it was shown that they had (*i*) stable species numbers from 1917 to 1968, as a result of (*ii*) a dynamic equilibrium between immigration and extinction (Diamond, 1969), and (*iii*) that this equilibrium was the result of competition due to niche limitation (Jones & Diamond, 1976). Second, the lizard genus *Anolis* on the Greater Antillean islands has had (*i*) a net diversification rate close to zero during the last few million years, with (ii) a balance between speciation and extinction (Rabosky & Glor, 2010), and (*iii*) a significant decrease in the rates of evolution for adaptive traits, suggesting niche limitation (Mahler *et al.*, 2010).

From these and other examples included herein, it is evident that the different aspects of species saturation have mainly been assessed using insular systems, where studying saturation is easier than in mainland settings because of the well-defined, replicated study units. These examples illustrate further the need for long-term monitoring studies in different mainland and island contexts, and the synergistic advantages of combining results from different data sources in ecology and evolution. Clearly, many more such inclusive studies are necessary before we can reach any general conclusions about the occurrence of saturation in species assemblages.

V. CONCLUSIONS

(1) Studies must provide both conceptual and operational definitions of saturation as it applies to their study.

(2) We propose a conceptual definition of saturation: *saturation is the dynamic equilibrium of species richness due to niche limitation within a given group of organisms and within an environmental and temporal reference frame*.

(3) Operationally, our definition of saturation implies that for saturation to be demonstrated we need to show (*i*) stability and/or, (*ii*) equilibrium, both under the condition that other species must be able to immigrate or originate, and (*iii*) that a niche-limitation mechanism underlies either stability or equilibrium.

(4) In a saturation assessment, the reference system (e.g. spatial and temporal scales) should be clearly defined because saturation might or might not be found in a given system depending on the references considered.

(5) A major unsolved challenge is deciding if fluctuations in the number of species reflect stability or not. Thus, depending on the study system, authors should define *a priori* how stability is defined and how variability is dealt with.

(6) It may be more informative to consider saturation not as a binary concept (saturated or not) but as a ratio (e.g. 80% saturated) as proposed for bounded diversity by Cornell (2013).

(7) Despite a number of publications on the topic of species saturation, most studies conducted to date have ignored one or more of the above challenges, so that our understanding of the prevalence of saturation in natural systems is still very limited.

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Chapter 2

Determinants of Beta Diversity Across Different Spatial Scales

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vi. ABSTRACT
Aim: To explore how local and regional factors influence the geographical change in species composition (β diversity) at each scale and across them.

Location: Bolivia.

Major taxa studied: Ferns.

Methods: 1227 plots were established to inventory fern species and local environmental conditions. We assessed the influence of species richness, environment, and geographical distance on β diversity of ferns within and across scales mainly via Mantel tests.

Results: Variations in species richness and environmental factors were the strongest predictors of β diversity within both local and regional scales. Across scales, we found that local β diversity was also predicted by variation in regional environmental factors but not by variation in the regional number of species. Regional β diversity was predicted by variation in local species richness and to a lesser degree by local geographical distances, but not by variation in local environmental conditions.

Main conclusions: Our findings contrast with those from nearby lowland regions where geographical distance commonly has the strongest influence. This highlights the importance of environmental heterogeneity on the distribution and evolution of biodiversity in mountain ecosystems and, suggests that an effective conservation strategy in mountains should put a strong focus on habitat heterogeneity.

Key words: biodiversity, ferns, Mantel, Neotropics, plots, beta diversity, elevational transects

vii. MAIN TEXT

INTRODUCTION

Understanding how biological diversity is distributed in space is one of the core aspects of research in ecology, evolution, and conservation. Much of this understanding is the result of the simplest type of measurement: counts of species numbers. However, besides counts of local or regional species numbers (α and γ diversity), the geographical change in species composition (β diversity) is also relevant to understand the spatial distribution of diversity. β diversity can be expressed as a rate or as a distance (Whittaker, 1960, 1972). In the former case, β diversity is considered as the fraction of the total (γ) diversity distributed among local sites (α). In the latter case, β diversity is treated as a dissimilarity in species composition between sample units (Anderson *et al*., 2011; Tuomisto, 2010a; Whittaker, 1972).

β diversity reflects the variation in species arrangements. Thus, areas with high levels of β diversity typically have small, patchily distributed species populations as well as concentrations of endemic and restricted-range species, rendering them important targets for conservation. Mountains typically have higher levels of β diversity than lowland areas at both regional (Kessler, 2000; Kessler, Parris, & Kessler, 2001; Mourelle & Ezcurra, 1997) and continental (McKnight *et al*., 2007) scales. This is commonly linked to the great variety of habitat conditions which render mountain ecosystems vital for the generation and maintenance of biodiversity (Antonelli, 2015; Fjeldså, Bowie, & Rahbek, 2012; Hoorn, Mosbrugger, Mulch, & Antonelli, 2013; Hughes & Atchison, 2015; Wang, Schneider, Zhang, & Xiang, 2012), especially under climate change conditions (Sandel *et al*., 2011). Unfortunately, the enormous diversity of many mountain areas is currently threatened by long-lasting and ongoing destruction of wild habitats (N. Myers, Mittermeier, Mittermeier, Fonseca, & Kent, 2000). Mountain ecosystems are therefore in urgent need for conservation strategies, whose effectivity depends on the understanding of β diversity patterns (Socolar, Gilroy, Kunin, & Edwards, 2016).

β diversity is determined by numerous factors, including the biology of the studied organism (e.g. motile vs sessile, Kessler, 2000), its interspecific interactions (Callaway *et al*., 2002; Wardle, 2006), the sampling design (size, number and arrangement of samples, (Kraft *et al*., 2011; Qian, Wang, & Zhang, 2012; Tuomisto & Ruokolainen, 2012), geographical region (e.g. temperate vs tropical areas, (McKnight *et al*., 2007; Qian & Ricklefs, 2007), and the environmental gradients associated to it (e.g. temperature,(Fitzpatrick *et al*., 2013); humidity,(Jankowski, Ciecka, Meyer, & Rabenold, 2009; Karst, Gilbert, & Lechowicz, 2005; Richard, Bernhardt, & Bell, 2000); soils, Tuomisto *et al.* 2002, 2003; productivity, (Harrison, Davies, Safford, & Viers, 2006); as well as species richness, Jost 2007, 2010, Tuomisto 2010a, De Cáceres *et al*. 2012, Ulrich *et al*. 2017). The influence of geographical distance can be the result of environmental gradients (Nekola & White, 1999) or of variation in the dispersal capacity of the species (Tuomisto, Ruokolainen, *et al*., 2003). Environmental factors in turn influence β diversity directly because different species have different ecological requirements (Chapin, Bloom, Field, & Waring, 1987; Jones, Szyska, & Kessler, 2011; Krömer, Kessler, & Gradstein, 2007; Silvertown, 2004; Svenning, 2000). Nevertheless, the strength of this relationship may vary at different parts of an environmental gradient (Callaway *et al*., 2002; Huston, 1999).

It is well established that both geographical distance (as a proxy for dispersal) and environmental variation are main drivers of species turnover in biotic communities, and the question is which of these factors dominates under which conditions. For example, (Myers *et al*., 2013) argued that the importance of these factors in explaining β diversity of plants differs along the latitudinal gradient, with environment being more important in temperate areas and geographical distance in tropical areas. Nevertheless, studies in tropical areas have found these factors to be of different importance depending on the amount of environmental variation. For instance, a study in eastern Amazonia found geographical distance to be the most important driver of β diversity (Tuomisto *et al*., 2003) whereas in western Amazonia, where there is higher topographical structure and therefore higher environmental heterogeneity (Hoorn *et al*., 2010), β diversity correlated more to environmental factors like inundation regime and soils (Draper *et al*., 2017; Kristiansen *et al*., 2012). Hence, in mountainous regions, where large environmental heterogeneity occurs in relatively small extents, it is likely that the environment plays a more important role in explaining β diversity than geographical distance (Jankowski *et al*., 2009; Kessler, 2000).

Changes in species richness can also influence β diversity in two ways. First, if sampling units are so small that they only include a small percentage of the regional species pool, then β diversity between sampling units will be high because it is less likely that two samples will include the same species. The opposite occurs if the samples are so large that each of them contains most species from the regional pool (Jost, 2007; Karger *et al*., 2015; Tuomisto, 2010b). Second, if sampling units have a significant variation in species richness independently from their size, we expect β diversity to vary

accordingly, simply because communities of different richness by default cannot be identical. Previous evidence suggests that β diversity varies along gradients of species richness (McKnight *et al*., 2007; Qian & Ricklefs, 2007). However, this effect may simply be a statistical artefact, and separating the statistical from the ecological patterns remains a challenge (Baselga, 2007).

In addition, the influence of all of the above factors changes with spatial scale, and therefore to fully understand the factors determining it, β diversity must be assessed at different extents and grain sizes (Karger *et al*., 2015; Soininen, McDonald, & Hillebrand, 2007; Whittaker, 1972), as various studies have done (Condit *et al*., 2002; Harrison *et al*., 2006; Karger *et al*., 2015; McKnight *et al*., 2007; Myers *et al*., 2013; Novotny *et al*., 2007; Qian & Ricklefs, 2007; Tuomisto *et al*., 2003). Within these studies, the drivers of β diversity are typically studied at a given scale, so that, e.g., regional-scale factors are used to explain regional-scale β diversity. But cross-scale effects are also known to be potentially important. For example, β diversity at a local scale changes over a regional scale with elevation (McKnight *et al*., 2007; Melo, Rangel, & Diniz-Filho, 2009), temperature, and the size of the regional species pool (Mourelle & Ezcurra, 1997; Tello *et al*., 2015). Conversely, local environmental filtering and competition might influence patterns of β diversity at regional scales (Sabatini Francesco, Jiménez-Alfaro, Burrascano, Lora & Chytrý, 2017). Cross-scale factors have, however, received limited attention.

To exemplify the complexity of these cross-scale influences, we can consider a hypothetical study of a series of vegetation plots established at different elevations along an elevational gradient. We can calculate β diversity between plots at a given elevation (β_{within}) as well as between elevational bands along the overall gradient ($\beta_{between}$). We can try to explain β_{within} by plot-level factors, such as ecological conditions of the plots or the spatial distances between them. Similarly, we can attempt to explain $\beta_{between}$ by the ecological differences between bands and their distances. If we now turn to the cross-scale effects, β_{within} may also show a relationship to factors changing along the elevational gradient. For instance, if habitat heterogeneity increases with elevation, then β_{within} will also show an increase with elevation. Importantly, this will not necessarily be evident if we study the explanatory effect of habitat heterogeneity separately for each elevational band because if both β_{within} and heterogeneity increase in parallel, then the explanatory effect of heterogeneity within bands will not change with elevation. In addition, $\beta_{between}$ may also be influenced by factors within belts. For instance, high β diversity within bands leads to higher overall species numbers in each band, leading to greater species overlap between bands and hence lower $\beta_{between}$ diversity.

In the present study, we explored how local and regional patterns of species richness, environmental factors and geographical distance influence β diversity at local and regional scales, and across both scales. We did so by analysing a data set of 1227 plots established in Bolivia that contains 916 fern species (77.3% of the Bolivian fern flora, Kessler & Smith, 2017) and covers the main forest types and climatic variation of the country (Fig. 1). Ferns are a suitable group of plants for this kind of study because they occur with fairly high species numbers across a wide range of forest habitats (Kessler, 2002; Kessler, Kluge, Hemp & Ohlemüller, 2011; Salazar *et al*., 2015), have a reasonably well-known taxonomy (PPG I 2016, Kessler & Smith, 2017), and because their spore dispersal reduces the effects of stochastic dispersal limitation (Barrington, 1993; Linares-Palomino & Kessler, 2009). We included three groups of determinants: species richness, environmental conditions, and geographical distance.

We developed a set of 11 testable hypotheses (Table 1). In H1-3 we explored the relationship between local β diversity (β_L) and the variation in local fern species richness (R_L), local environment (E_L), and geographical distance between plots (D_L). We hypothesized that variation of all local factors will have a positive effect in β_L. Hypotheses H9-11 are analogous to those in H1-3 but at the regional scale. In hypotheses H4-8 we explored the determinants of β diversity patterns across scales. In H4-5 we expected variation in R_R to explain part of the variation in β_L but less than the fraction explained by variation in E_R. We do not conceive how regional geographical distance (D_R) could have a causal influence on β_L, and therefore no test was performed. Finally, in H6-8 we expected increasing variation in R_L and E_L to lead to increasing values of β_R, whereas we did not expect an influence of D_L.

METHODS

We used a large dataset of 1227 plots fully described in Kessler (2000) and Salazar *et al*. (2015), distributed across 62 sites in Bolivia (Fig. 1a).

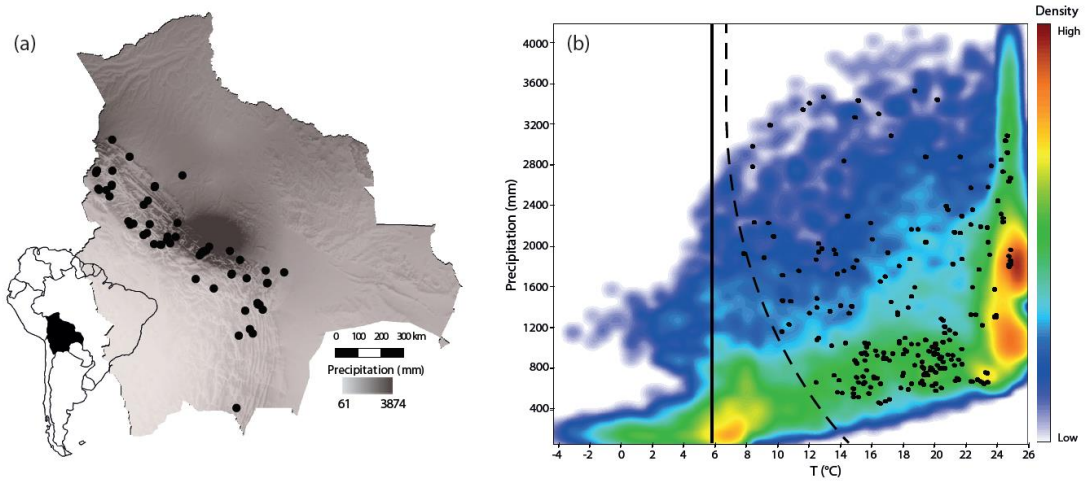

Figure 1. Map of Bolivia showing the locations of the 62 sites (a). Distribution of plots in a density diagram showing the climatic conditions (mean annual temperature vs. mean annual precipitation) of Bolivia (b). The solid line shows the approximate climatic limit of the tree line following (Körner & Paulsen, 2004), and the dashed line shows the current limit of the tree line mainly determined by human disturbance (Fjeldså, Kessler, Engblom, & Driesch, 1996). Note that the plots cover most of the available climatic space within the range of forest biomes.

Sites ranged from 200-3950 m in elevation, and 400-3500 mm in mean annual precipitation, thus covering most climatic conditions where forests occur in Bolivia (Fig. 1b). Sites were placed in forest biomes and we did not sample non-forest biomes (savannahs or alpine vegetation) because ferns are very poorly represented in them. The number of plots at each site ranged from 9 in species-poor to 44 plots in the more diverse areas (mean = 21 plots). Sites covered different elevational ranges (0-2000 m; mean = 570 m), and varied habitat conditions including rock faces, ravines, secondary forests and primary forests.

Each plot was 400 m^2 (usually 20 m x 20 m) in size. In each plot, all fern species (terrestrial and epiphytic) were identified and counted, and environmental information on elevation, topography, aspect, epiphytic moss cover as a proxy for air humidity (Karger *et al*., 2012), and canopy openness was recorded (Table 2). Voucher specimens were collected and deposited in the herbaria in La Paz (LPB), Göttingen (GOET), and Berkeley (UC). Individuals were classified to the species level, and nomenclature was unified according to (PPG I, 2016) and (Kessler & Smith, 2017) based on two decades of taxonomic work on Bolivian ferns by M. Kessler and A.R. Smith (UC Berkeley). Unidentified individuals (<4%) were excluded from analyses. In total, our dataset contained information on the distribution of 23029 occurrences (plot-records) of 916 species.

Table 1. Hypotheses tested in this study. H1-H3 focus on the relationships at the local scale. Analogous relationships at the regional scale are examined in H9-11. H4-5 focus on the relationships of regional factors to β_L and H6-8 of local factors β_R. We consider that regional geographical distance (D_R) cannot have a causal influence on β_L, and therefore no hypothesis is tested in this regard. Symbols (-- to ++) indicate the expected relative directions and strengths of the relationships.

	Local factors			**Regional factors**		
	Species richness (R_L)	**Environment (E_L)**	**Distance (D_L)**	**Species richness (R_R)**	**Environment (E_R)**	**Distance (D_R)**
	H1	***H2***	***H3***	***H4***	***H5***	
Local β diversity (within localities) **β_L**	(+) β_L increases with increasing difference in species richness between plots	(++) β_L increases with increasing environmental heterogeneity between plots	(+) β_L increases with increasing distance between plots	(+) β_L increases with increasing regional species richness	(+?) β_L may vary systematically with environmental factors such as elevation	no causal effect conceivable

	H6	*H7*	*H8*	*H9*	*H10*	*H11*
Regional β diversity (between localities) **β_R**	(?) Perhaps sites with more species have lower β_R because of greater species overlap	(?) Perhaps sites with greater internal variability have lower β_R because they have greater species overlap	(--) none expected	(+) β_R increases with increasing difference in species richness between sites	(++) β_R increases with increasing environmental heterogeneity between sites	(+) β_R increases with increasing distance between sites

Data analysis

β *diversity estimates*

We defined β diversity as the dissimilarity in species composition between sampling units. We used plots as our sampling units at the local scale (Fig. 2a) and sites as the units at the regional scale (Fig. 2d).

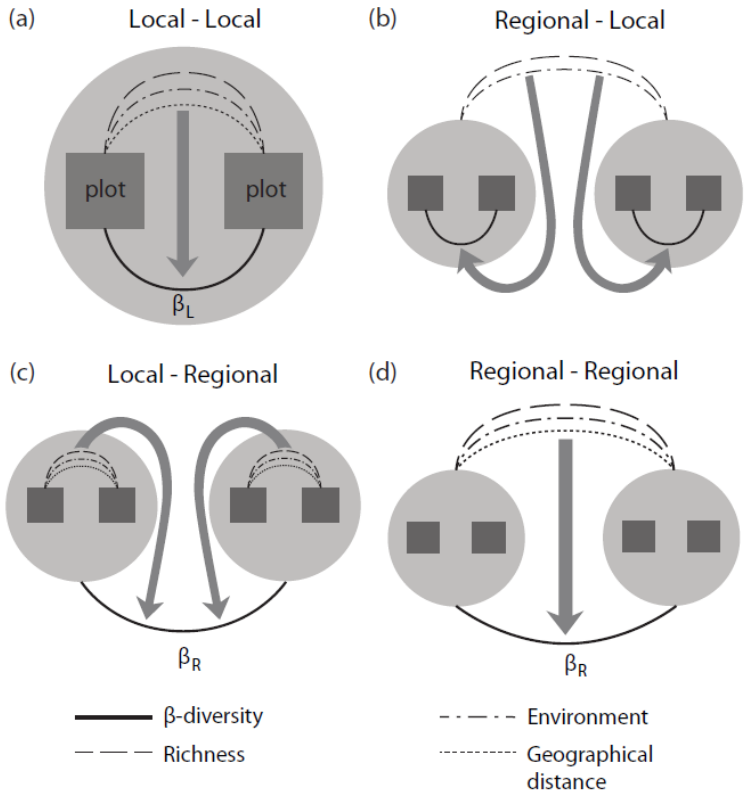

Figure 2. The four combinations of scales that may influence measures of β diversity and the 11 hypothesis that were tested in this study. We used plots (squares) as sampling units at the local scale, and sites (circles) as sampling units at the regional scale. At each scale, we calculated Euclidean dissimilarity matrices in species composition (β diversity), species richness, and environmental and geographical distances. Four main types of relationships were assessed, **(a)** and **(d)** test for single-scale relationships: **(a)** the influence of between-plot variation in species richness, environment, and geographical distance on local β diversity; and **(d)** the same at the regional scale. **(b-c)** test for cross-scale relationships: (**b**) the influence of regional factors (except distance) on local β diversity, and **(c)** the influence of local factors on β_R. See text for details and Table 1 for a description of the hypotheses.

a) Local β *diversity* (β_L)

We defined β_L as the dissimilarity in species composition between plots belonging to the same site (Fig. 2a, b). We first calculated the Sørensen similarity index for incidence-frequency data in Estimate*S*

(Colwell, 2013) and then obtained dissimilarity values by subtracting each matrix from the unit. Because plots were assumed to have been fully sampled (i.e., no missing species), we did not correct β diversity values for sampling incompleteness.

b) Regional β *diversity* (β_R)

We defined β_R as the dissimilarity in species composition between sites (Fig. 2c, d). To account for differences in sampling intensity (number of plots), we used the Chao-Sørensen similarity index (Chao, Chazdon, Colwell, & Shen, 2005) to correct for unseen species that could bias β diversity estimates (Jost, 2010). We first calculated the Chao-Sørensen similarity index for incidence-frequency data in Estimate*S* and then obtained dissimilarity values by subtracting each matrix from the unit. Next, because numerous sites had no species in common, we recalculated the dissimilarity matrix between sites using the "*stepacross"* function in the R package vegan (Oksanen *et al*., 2017). This approach essentially searches for the closest "path" between two sites using intermediate sites (Zuquim *et al*., 2012).

Species richness

a) Differences in local species richness (R_L)

We calculated a dissimilarity matrix based on the total observed number of species in each plot (R_L).

b) Differences in regional species richness (R_R and R_{Nsp})

We estimated the number of species in each site as the average between the Chao2 and MMMean estimators calculated in Estimate*S.* These estimators have low sensitivity to variation in species pool size and sample size, respectively (Herzog, Kessler, Cahill, & Hackett, 2002). In our subsequent analyses, we used the estimated species richness per site (R_{Nsp}) or the dissimilarity in species richness between sites (R_R).

Environmental factors

We assembled a dataset with information at the plot level including variables collected in the field, and climatic values extracted from BIOCLIM V1.1 layers of the CHELSA model (Karger *et al*., 2017).

a) Differences in local environmental conditions (E_L)

We used the "*findCorrelation"* function in the R package *caret* (Kuhn, 2008) to eliminate all variables with a correlation value higher than 0.7. We then calculated individual environmental dissimilarity matrices between plots within each site using the ten remaining variables at the local scale (Table 2, columns i-x).

b) Differences in regional environmental conditions (E_R)

For the regional analyses, we first averaged the environmental factors for all plots in each site, then eliminated all variables with a correlation value higher than 0.7 also using the "*findCorrelation"* function. This procedure resulted in eight variables (Table 2 columns iv-viii, x-xii) that we used to calculate a single environmental dissimilarity matrix for the whole region.

Table 2. Environmental variables recorded in each plot (i-viii) and extracted for each plot from CHELSA-BIOCLIM V1.1 (ix-xii).

	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii
Variable name	N-S aspect	E-W aspect	Habitat	Elevation	Moss cover	Inclination	Plant cover	Canopy cover	Mean annual temperature	Mean annual precipitation	Temperature seasonality	Precipitation seasonality
Description or units	Latitude aspect: N=1, S=-1	Longitude aspect: E=1, W=-1	1: rock face, 2: ridge, 3: lakeshore, 4: ravine, 5: swamp forest, 6: grassland, 7: scrub, 8: plantation, 9: secondary forest, 10: zonal forest	Meters above the sea level (m)	Estimated % of branches covered by mosses	Degrees relative to the horizontal plane (°)	Estimated % of ground covered with plants	Estimated % of canopy cover	(°C)	(mm/year)	Annual standard deviation	Annual coefficient of variation

Geographical distance

We calculated geographical distance dissimilarity matrices between plots *a)* within each site (D_L) and *b)* a single distance matrix for the geographical distance between sites (D_R), using the average location of all plots within a site as site location.

Testing hypotheses

Our hypotheses can be divided in four general sets. The first studies how β_L correlates to the variation in local factors (Fig. 2a), the second set studies the relationship between regional factors and β_L (Fig.

2b), the third one studies the relationships between local factors and β_R (Fig. 2c), and the last one focuses on how β_R correlates to the variation in regional factors (Fig. 2d).

Mantel and partial Mantel tests

We used Mantel tests to calculate the relationship between β diversity and species richness, environment, and geographical distance and partial Mantel tests to estimate the relative influence of each factor by controlling the correlations between them at both scales (Table 3). Following (Legendre, Borcard, & Peres-Neto, 2005) we used Mantel tests because our aim is to compare the relative correlations between β diversity and its predictors among groups of plots and sites. In the partial mantel tests, the correlation between matrices *y* and *z* (e.g., E_L and D_L) is partialled out before calculating the correlation between matrices *x* and *y* (e.g., β_L and E_L). The standardized form of the Mantel statistic (r_M) was used, which is equivalent to the Pearson correlation coefficient between two dissimilarity matrices. Statistical significance was established at the *P*<0.05 level with a Monte Carlo permutation test using 999 permutations. We performed two partial Mantel tests for each factor (against both other factors) and calculated the average of the two r_M values as the final correlation coefficient for each of these factors (Supplementary material Appendix 1, Table A1).

Table 3. Relationships between β diversity and species richness, environment and geographical distance, and the relative importance of each factor at both scales (local and regional). In all partial Mantel tests the correlation coefficient ($\bar{r}_M$) is the average correlation between the two tests performed for each factor (see Table 3). Additionally, in (a) the correlation coefficients are the average for all 62 sites; here the number of sites for which the correlation was significant at p<0.05 (*****) is indicated for each hypothesis. In b-d significance follows: *****p<0.05, ******p<0.01, *******p<0.001. See Supplementary Material Appendix 1, Tables A2-A5 for the specific results of each test.

	(a) β local vs local factors			**(b) β local vs regional factors**		
	H1	**H2**	**H3**	**H4**	**H5**	
	Species richness	**Environment**	**Geographical distance**	**Species richness**	**Environment**	**Geographical distance**
Mantel	$\bar{r}_M$=0.34 ***** 47 sites	$\bar{r}_M$=0.34 ***** 48 sites	$\bar{r}_M$=0.15 ***** 27 sites	r_M=0.01	r_M=0.19******	
Partial Mantel	$\bar{r}_M$=0.32 ***** 46 sites	$\bar{r}_M$=0.31 ***** 42 sites	$\bar{r}_M$=0.07 ***** 16 sites	$\bar{r}_M$=0.01	$\bar{r}_M$=0.16*****	
	(c) β regional vs local factors			**(d) β regional vs regional factors**		
	H6	**H7**	**H8**	**H9**	**H10**	**H11**
Mantel	r_M=0.37*******	r_M=0.05*******	r_M=0.09*****	r_M=0.28*******	r_M=0.27*******	r_M=0.09******
Partial Mantel	$\bar{r}_M$=0.37*******	$\bar{r}_M$=0.03	$\bar{r}_M$=0.09*****	$\bar{r}_M$=0.28*******	$\bar{r}_M$=0.27*******	$\bar{r}_M$=0.005

To test for correlations between β_L and regional factors (Table 3b), we calculated the average dissimilarity value for β_L within each site and calculated a new dissimilarity matrix: average dissimilarity in β_L or ($\bar{\beta}_L$). Then we performed partial Mantel tests between ($\bar{\beta}_L$) and R_R as well as E_R. To test for correlations between β_R and local factors (Table 3c), we calculated the average dissimilarity value for each factor (R_L, E_L, and D_L) within each site and calculated a new dissimilarity matrix for each factor: average dissimilarity in local species richness ($\bar{R}_L$), average dissimilarity in local environment ($\bar{E}_L$), and average dissimilarity in local geographical distance ($\bar{D}_L$). We then performed partial Mantel tests between different combinations of these average local dissimilarity matrices and the dissimilarity matrix for β_R. All Mantel tests were calculated using the "*mantel"* and "*mantel.partial"* function in the R package *vegan* (Oksanen *et al*., 2017).

We illustrated the results from the partial Mantel tests with correlograms using the package *ggplot2* (Wickham, 2009). To do this we plotted lines representing the general trend in each of the correlations based on linear models. In each case, we evaluated whether linear or log regression was a better fit to the data using Akaike Information Criterion (AIC) and plotted the resulting model.

Individual Mantel tests for environmental factors

We also performed Mantel tests to evaluate the correlation between each environmental factor and the average beta diversity at the local sites as well as in the whole region. By doing so, we expect to discriminate between the factors most strongly correlated with beta diversity patterns at each scale.

Influence of sampling completeness at the site level

We used regression analyses to test if the fact that some sites contained a larger proportion of the regional species pool affected the patterns of β diversity and their relation to species richness, environment and geographical distance. To do this, we calculated regression models between the average partial correlation coefficient r_M obtained for the correlation between β_L and each factor (R_L, E_L and D_L) against the percentage of species from the regional pool recorded on average in the plots of each site.

Finally, to discard that possibility that our results were merely an artefact of the large environmental variation covered by our sampling, we re-ran all our analysis using a subset of data that contained only plots located in zonal forests (758 plots). Also, to assess the influence of life forms, we divided the data set by terrestrial and epiphytic species. All these results did not differ qualitatively from those obtained using the complete dataset and therefore are not shown.

RESULTS

Mantel and partial Mantel tests

At the local scale, both the Mantel and partial Mantel tests showed that the correlations between β_L and local species richness and local environment were significant (at $p<0.05$) in most of the sites (Table 3a). In contrast, geographical distance between plots was significantly correlated to β_L in fewer sites and with a lower correlation coefficient, in particular when its effect was partialled out from the interaction to the other factors. Relative to regional factors, β_L was correlated to variation in regional environmental conditions but not to variation in regional richness (Table 3b, Fig. 3).

At the regional scale, β_R was significantly correlated with differences in regional richness and regional environment in a similar proportion, but there was no significant correlation to the geographical distance between sites (Table 3d). Relative to local factors, β_R was significantly correlated with variation in local richness and to a lesser extent to local geographical distance, but not to local environment (Table 3c, Fig. 3).

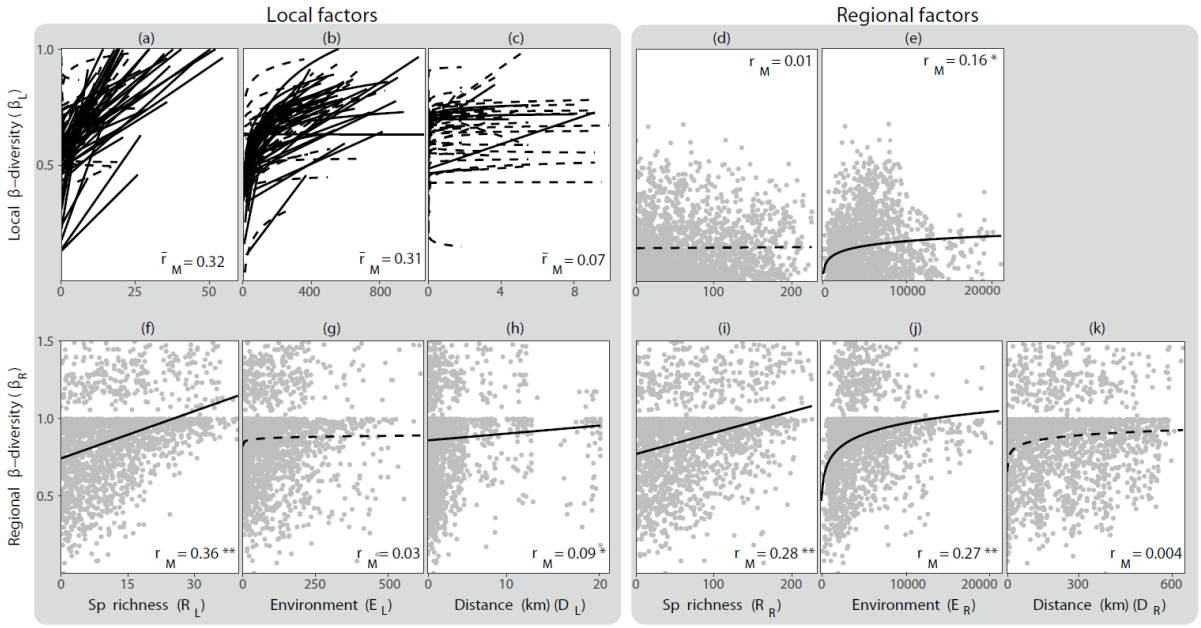

Figure 3. Correlograms of local (β_L) and regional (β_R) β diversity of ferns in relation to local ($_L$) and regional ($_R$) distances in species richness (R), environment (E), and geographical distance (D). Lines representing the general trends were drawn based on linear models. Correlation coefficients ($\bar{r}_M$) correspond to the average correlation coefficient obtained from the two partial Mantel tests performed for each factor, the significance level (*<0.05, **<0.01) corresponds to the highest p-value obtained for each factor. Gray dots represent individual distances between sites. Distances between plots are not shown in panels (a)-(c) because it would be impossible to visually relate them to the individual trend lines. In these panels, the average correlation coefficient of all 62 localities is given, but no significance level can be indicated. Individual Mantel tests were significant (at $p<0.05$) for 49 (out of 62) sites in (a), 51 sites in (b), and 31 in (c). The top right panel remains blank as it is not ecologically meaningful to test for the effect of regional geographical distances on local β diversity.

The similarity in the results from both Mantel and partial Mantel tests, particularly at the regional scale, reflected the limited correlation in our dataset between richness and environmental factors (r_M=-0.03, p=0.7) and between richness and geographical distance (r_M=0.024, p=0.2). In contrast, variation in environmental factors between sites was correlated to geographical distance (r_M=0.56 p=0.001). Specific results for each of the 62 sites and for all hypotheses is provided in the Supplementary Material Appendix 1, Tables A2-A5.

Individual Mantel tests for environmental factors

The environmental factors that significantly correlated to variation in β_L were the differences in habitat and the variation in ground plant cover (Table 4). At the regional scale, the factors that correlated most strongly to variation in β_R were elevation, moss cover on branches, and mean annual precipitation.

Table 4. Correlation coefficients for specific Mantel tests between the average $\bar{\beta}_L$ and local environmental factors as well as β_R and the corresponding regional environmental factors. ******p<0.01, *******p<0.001.

Factor	β_L	β_R
N-S aspect	0.05	-
E-W aspect	0.05	-
Habitat	0.20**	-
Elevation	0.00	0.50***
Moss cover	-0.08	0.23***
Inclination	0.01	0.12**
Plant cover	0.15**	0.10**
Canopy cover	-0.04	0.16***
Mean annual temperature	0.00	-
Mean annual precipitation	-0.07	0.17***
Temperature seasonality	-	0.17***
Precipitation seasonality	-	0.12**

Influence of sampling completeness at the site level

β_L declined logarithmically with the increase in the percentage of the estimated total number of species of a site recorded on average in the plots of that site (R^2=0.2802, p=5.8^{e-6}, Fig. 4a). However, this percentage had no influence on the strength of the correlation (r_M) between β_L and local species richness (R^2=-0.02, p=0.93, Fig. 4b), environment (R^2=0.00, p=0.39, Fig. 4c), or geographic distance (R^2=0.00, p=0.27, Fig. 4d).

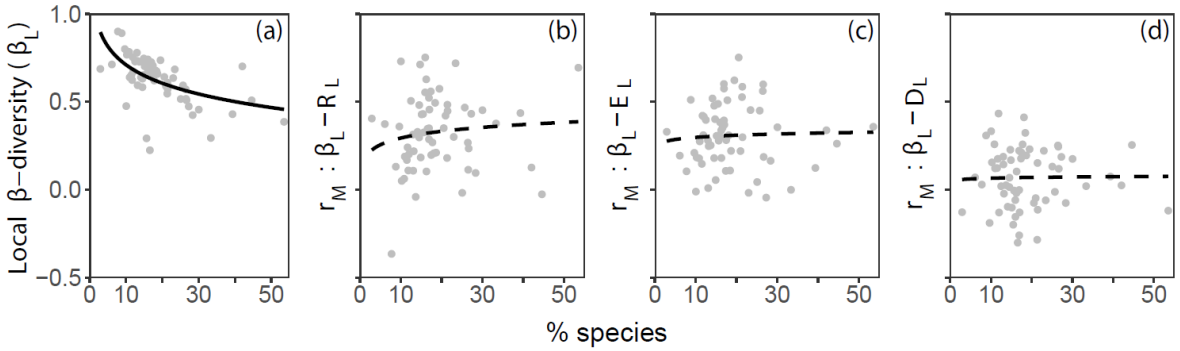

Figure 4. Influence of the average proportion (% species) of the estimated regional species pool recorded on average in each plot of each site on estimates of β_L and its relation to variation in local factors. The solid line represents a significant regression model at p<0.05, dashed lines are non-significant.

DISCUSSION

The main results of our study can be summarized as follows: (1) variation in species richness and environmental factors were the strongest predictors of β diversity at both local and regional scales, (2) β_L was also well predicted by variation in regional environmental factors, and (3) β_R was also predicted by variation in local species richness (R_L) and to a lesser degree by local geographical distances (D_L).

Our results thus confirm the expectations based on previous studies (Kessler, 2000; Kessler *et al*., 2001; McKnight *et al*., 2007; Mourelle & Ezcurra, 1997; Tello *et al*., 2015) that spatial distance becomes less relevant in mountain settings than in lowlands because of the high heterogeneity of local conditions within relatively short distances. This effect was almost identical at both scales of our study. In addition, we also found strong cross-scale correlations, although here different factors were involved at the different scales.

Overall, the variance of species composition captured by the factors species richness, environment, and geographical distance, with r-values of up to 0.37 for individual factors, is comparable to that reported in previous studies of ferns (Karst *et al*., 2005; Richard *et al*., 2000; Tuomisto, *et al*., 2003). The remaining uncorrelated variation might reflect the lack of data on important environmental variables (Harrison *et al*., 2006; Zuquim *et al*., 2012), biotic interactions such as competition or facilitation between ferns as well as with other plants or animals (Callaway *et al*., 2002; Wardle, 2006), and stochastic variation (De Cáceres *et al*., 2012; Karst *et al*., 2005). For example, studies in Ecuador have shown that around half the fern species in plots of 400 m^2 are only represented by a few sterile individuals, most likely as the result of sporadic colonization events that are unlikely to result in persistent populations in a given plot (Kessler, Salazar, Homeier, & Kluge, 2014) but that can greatly influence the perception of diversity patterns (Kessler *et al*., 2011). In our study, we were unable to decide which species in a plot would belong to the "core" flora and which ones were only transient "visitors", so we were unable to assess the potential influence of the latter.

The influence of variation in species richness

The effect of differences in local species richness on β_L and particularly on β_R is foremost a sampling effect (Baselga, 2007): two samples (plots or sites) with very different species richness cannot have high similarity, even if the species-poor sample is a subsample of the species-rich one. Thus, two samples can only have high similarity if they have similar species richness, although of course they can also be dissimilar. As a result, increasing differences in species richness between samples creates a "forbidden zone" of high similarity values that cannot occur. This effect has rarely been considered in previous studies of β diversity, so we are unable to compare our results with those of other regions or taxonomic groups.

Although we interpret the influence of species richness on β diversity as a sampling effect, there is also an underlying ecological causality. Spatial variation in species richness is common in ferns and many other organisms, and is often linked to environmental conditions (Hawkins *et al*., 2003). Thus, one may argue that the explanatory power of species richness also partly reflects the underlying ecological factors determining the species richness patterns. On the other hand, there is also considerable stochastic variation of species richness, especially at local scales (Stevens, Petchey, & Smouse, 2003). Previous studies on ferns have shown that adjacent plots of 400 m^2 under apparently identical environmental conditions typically differ by up to 30-50% in species richness (Karger *et al*., 2015; Kessler *et al*., 2011, 2014; Kluge, Kessler, & Dunn, 2006), which, as we show here, affects measures of β diversity.

We also found that β_L declined with an increasing percentage of species from the "regional pool" (sites) contained in local plots. This is an expected outcome because as more species are included in two samples, the potential for species overlap and hence higher similarity increases. Importantly, however, this had no effect when assessing the relation between β diversity and the explanatory variables. Thus, the variation of β diversity along the three gradients (species richness, environmental factors, geographical distance) was independent from the distribution of local species richness.

The influence of environmental factors

Our results are in line with previous studies showing that patterns of β diversity in mountains are strongly influenced by environmental conditions (Kessler, 2000; Kessler *et al*., 2001; McKnight *et al*., 2007; Mourelle & Ezcurra, 1997; Tello *et al*., 2015). This is the direct result of the high spatial variability of ecosystem conditions related to topographical factors such as elevation, aspect, inclination, and landscape arrangement (Antonelli, 2015; Fjeldså *et al*., 2012; Hoorn *et al*., 2013; Hughes & Atchison, 2015; Wang *et al*., 2012). In our study, the influence of local environmental factors was most strongly related to changes in the type and amount of vegetation cover. These mainly reflect the amount of light availability at the ground level, which is well known to influence the distribution of forest herbs in general (Svenning, 2000) and of ferns in particular (Kessler *et al*., 2014). For epiphytes, the size, age, and structure of the tree layer is of crucial importance (Krömer *et al*., 2007) and this varied between different habitats in our study. At the regional scale, elevation and climatic variables related to precipitation were the most important environmental factors, confirming the importance of climate in determining the distribution of fern diversity (Kessler *et al*., 2011; Kluge *et al*., 2006). A previous study has also shown

that local and regional scales are interrelated, in that at one of our study sites local plots with drier and warmer conditions were inhabited by species typically inhabiting higher elevations than those from more humid and cooler sites (Jones *et al*., 2011). The complex influence of topographically-determined environmental heterogeneity on species richness and turnover in the Andes has also been documented for cacti (Mourelle & Ezcurra, 1997) and birds (McKnight *et al*., 2007; Melo *et al*., 2009), among many other taxa.

That environmental factors did not show higher correlation coefficients with β_L and β_R than species richness might be explained by the fact that not all important environmental variables were included in our study. For instance, soil variation is a powerful predictor of floristic patterns in Amazonian forests (Tuomisto *et al*., 2003) and Andean forests (Jones *et al*., 2011), but we lack soil information for our plots. Furthermore, even for those factors that were included, we did not consider within-plot variation (e.g., canopy cover), so that again we lack part of the potentially crucial information.

The influence of geographical distance

Previous studies in the Amazonian lowlands (Tuomisto *et al*., 2003) and elsewhere (Qian & Ricklefs, 2007) have shown that geographical distance is often the most important factor in explaining β diversity at a range of spatial scales. This contrasts with our finding that environmental differences play a more important role than geographical distance at both local and regional scales. We interpret this result as reflecting the enormous climatic variation in the Bolivian Andes, with different climates often distributed in a checkerboard pattern, so that nearby sites can be environmentally very different, whereas distant sites can be similar (Kessler, 2000) (Fig. 1). While not surprising considering the different geographical settings of the Andes compared to Amazonia, our results emphasize the fact that the relative strength of environment and geographical distance in determining β diversity depends on the variation of these factors within the study area (Draper *et al*., 2017; Kristiansen *et al*., 2012; Rahbek, 1995). In addition, our study was restricted to a single country, so that geographical distances were small compared to the extent of the Andes. If we were to extend our study to cover a larger part of the Andes, then the influence of spatial distance would undoubtedly increase.

CONCLUSIONS

Along with numerous other studies, our results provide evidence for the importance of environmental heterogeneity in determining patterns of biodiversity and community composition in mountain settings. Environmental heterogeneity is perhaps the main reason why mountains constitute global centres for the generation and maintenance of biodiversity since it provides opportunities for the adaptation of organisms to varied habitat conditions (Fjeldså *et al*., 2012; Hoorn *et al*., 2013; Wang *et al*., 2012), leads to a complex geographical structure of more or less isolated species populations, allowing intraspecific divergence and adaptation (Hughes & Atchison, 2015), and reduces extinction rates by shortening the distances that species ranges must shift to keep track of their habitat conditions during periods of climatic change (Sandel *et al*., 2011). Unfortunately, the vast diversity and productivity of Andean highland ecosystems has turned them into human pantries. A long history of land exploitation has resulted in many of these ecosystems being highly threatened and in urgent need for effective conservation strategies (Myers *et al*., 2000). Our results suggest that in mountains habitat heterogeneity should be one of the main factors in deciding conservation priorities. While large conservation areas often contain many different ecosystems, and are valuable on their own right, covering the whole range of habitats and communities in complex mountain ecosystems will also require a set of smaller conservation units. Considering that many Andean ecosystems are naturally more or less fragmented, they should be more amenable to being managed in small conservation units than ecosystems that by nature cover large expanses.

viii. REFERENCES

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ix. DATA ACCESSIBILITY STATEMENT

Plot data are not publicly available, contact M. Kessler for details. Climatic data were downloaded from the CHELSA website: http://chelsa-climate.org/.

x. BIOSKETCHES

Ingrid Olivares is a PhD student at the University of Zurich. Her main interests are community ecology of tropical forests as well as the development of ecological concepts.

Michael Kessler is scientific curator of the Zurich Botanical Garden. His research is centred on the biotic and abiotic factors determining patterns of species diversity and the distribution of species, especially in the tropics.

Supplementary material Appendix 1

Table A1. Hypotheses tested using partial Mantel tests and regression analyses. Upper case letters represent the dissimilarity matrices calculated at local and regional scales. β: beta diversity; R: species richness dissimilarity; E: environmental dissimilarity; D: geographical distance. Matrices calculated at the local scale are indicated by the subscript $_{(L)}$, regional distance matrices are indicated by the subscript $_{(R)}$. See main text for details.

	a) β local vs local factors			**b) β local vs regional factors**		
	H1	**H2**	**H3**	**H4**	**H5**	
	Species richness	**Environment**	**Geographical distance**	**Species richness**	**Environment**	**Geographical distance**
Mantel	β_L x R_L	β_L x E_L	β_L x D_L	$\bar{\beta}_L$ x R_R	$\bar{\beta}_L$ x E_R	
Partial Mantel	β_L x R_L x E_L	β_L x E_L x R_L	β_L x D_L x E_L	$\bar{\beta}_L$ x R_R x E_R	$\bar{\beta}_L$ x E_R x R_R	
Mantel	β_L x R_L x D_L	β_L x E_L x D_L	β_L x D_L x R_L	$\bar{\beta}_L$ x R_R x D_R	$\bar{\beta}_L$ x E_R x D_R	
	c) β regional vs local factors			**d) β regional vs regional factors**		
	H6	**H7**	**H8**	**H9**	**H10**	**H11**
Mantel	β_R x $\bar{R}_L$	β_R x $\bar{E}_L$	β_R x $\bar{D}_L$	β_R x R_R	β_R x E_R	β_R x D_R
Partial Mantel	β_R x $\bar{R}_L$ x $\bar{E}_L$	β_R x $\bar{E}_L$ x $\bar{R}_L$	β_R x $\bar{D}_L$ x $\bar{R}_L$	β_R x R_R x E_R	β_R x E_R x R_R	β_R x D_R x E_R
Mantel	β_R x $\bar{R}_L$ x $\bar{D}_L$	β_R x $\bar{E}_L$ x $\bar{D}_L$	β_R x $\bar{D}_L$ x $\bar{E}_L$	β_R x R_R x D_R	β_R x E_R x D_R	β_R x D_R x R_R

Chapter 3

Ascogrammitis lehnertii (Polypodiaceae): A New and Dominant Understory-Species from a Diverse Community of Grammitid Ferns in the Andes of Ecuador

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Ascogrammitis lehnertii (Polypodiaceae): A New and Dominant Understory-Species from a Diverse Community of Grammitid Ferns in the Andes of Ecuador

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***Abstract*—**In the scope of pantropical studies aimed at understanding how the diversity of grammitid ferns (Polypodiaceae) has evolved and is maintained, we studied a diverse grammitid community in treeline elfin forests in eight study plots of 400 m² each at 3200 m on Cerro Toledo, Loja, southern Ecuador. We recorded a total of 7986 individuals of 16 grammitid species. *Ascogrammitis lehnertii* is here described as a new species. We also recorded *Mycopteris leucosticta*, a species previously believed to be restricted to the Chocó region of Ecuador. We found that the grammitid species were segregated by habitat into trunk-base and trunk specialists, which suggests that species coexistence in these diverse epiphytic communities is at least partly linked to habitat segregation. *Ascogrammitis lehnertii* and *M. leucosticta* were the second and third most abundant species, respectively, and both were mostly trunk-base species. The new species *A. lehnertii* differs from its congeners by the combination of its relatively broad lamina, (2–)3–4 cm wide, fertile leaves bearing 2 mm long reddish setae in and near the sori, and by bearing proliferous roots from which new plants emerge. Phylogenetic analyses of *atpß*, *rbcL, rps4*, *trnG-trnR*, and *trnL-trnF* DNA molecular sequences, using maximum likelihood, place *A. lehnertii* as sister to *A. cuencana* with strong support. Despite previous intensive collection efforts in Loja for the past 30 yr, all but one of the collections of this new species were made in the last three years. The discovery of *A. lehnertii* and the new record of *M. leucosticta* highlight the patchy distribution of some species in the Andes and the value of surveying habitats that potentially host unrecognized plant diversity. In order to facilitate further discovery, we provide a key to all species of *Ascogrammitis*.

***Keywords*—**Amotape-Huncabamba, endemic species, phylogenetics, puna, taxonomy.

The Amotape-Huncabamba Zone in southern Ecuador and northern Peru is a region of high biodiversity and endemism (Borchsenius 1997). The zone acts as a species corridor allowing for interchange between the northern and central Andean species, and the elevational depression of the zone also allows for interchange between the western and eastern cordilleras (Weigend 2002; Quintana et al. 2017). Simultaneously, at the regional level, it is a region with steep and rugged topography, and highly variable geology and climate that results in a landscape of varied and isolated vegetation types (Mandl et al. 2010). The region also has exceptional fern diversity (Kessler and Lehnert 2009a), with many new species being continuously described (e.g. Lehnert and Tejedor 2016; Tejedor and Calatayud 2017). This also applies to the grammitid ferns, a monophyletic lineage of mostly epiphytic ferns belonging to the family Polypodiaceae (Ranker et al. 2004; Schneider et al. 2004; Sundue et al. 2015), which includes some 400 species in the Neotropics and 500 species in the Palaeotropics (Bauret et al. 2017). Previous studies have documented exceptionally rich communities of grammitid ferns in the Amotape-Huncabamba Zone, especially in stunted, mist-shrouded elfin forests on ridges, where up to 20 species of grammitid ferns can be found growing side by side (Kessler and Lehnert 2009a, b; Mandl et al. 2010; M. Kessler et al. unpubl. data). How this diversity has evolved and is maintained remains, however, poorly explored. In particular, we know little about habitat segregation among species of grammitid ferns and the potential role of interspecific competition in structuring grammitid communities. Because epiphytic habitats are highly dynamic due to tree growth and damage, it has been argued that epiphytic plant communities experience little competitive pressure (Benzing 2008; Zotz 2016). In order to understand community assembly processes among grammitid ferns, over the last few years we have conducted detailed field surveys in southern Ecuador. We here describe the grammitid community at Cerro Toledo and report the discovery of a new species of *Ascogrammitis* Sundue as well as of a population of *Mycopteris leucosticta* (J. Sm.) Sundue, a species previously restricted to the Ecuadorian Chocó (Sundue 2014).

Materials and Methods

***Study Site*—**Cerro Toledo is a mountain located in the southwestern corner of Podocarpus National Park in southern Ecuador, which encompasses cloud forests and páramo vegetation at 2300–3300 m elevation. The location and topography of this area produce extreme climatic conditions of high precipitation (up to 6000 mm per year) and high wind speed (Brunschön and Behling 2009). Despite (or perhaps partly because of) these harsh environmental conditions, of the 52 endemic plant species known from the park, Cerro Toledo was found to harbor the highest percentage of these in sampled plots (Lozano et al. 2010). Thus, there is an endemic flora that seems to benefit from the high rate of species migration and also from the intricate topography of the area that provides numerous microhabitats (Homeier et al. 2008).

Our sampling took place at 3200 m in the treeline ecotone where vegetation is characterized by patches of dwarf or elfin forest intermixed with tall, shrubby páramo vegetation. Most of the trees are small and form patches of forest with a canopy at 3–4 m height, but a few tall trees (up to 10 m height) from the cloud forest are still present. Common species belong to the genera *Blechnum* L. (Blechnaceae), *Gaultheria* L. (Ericaceae), *Loricaria* Wedd. (Asteraceae), *Meriania* Sw. and *Miconia* Ruiz & Pav. (Melastomataceae), *Myrsine* L. (Myrsinaceae), *Oreopanax* Decn. & Planch. (Araliaceae), and *Podocarpus* L'Hér. ex Pers. (Podocarpaceae), among many others (Lozano 2002).

***Grammitid Diversity*—**In July 2014 we sampled 16 plots of 20 3 20 m² at 3200 m on Cerro Toledo. In each plot, we recorded the abundance of all grammitid species at four different heights (zones) of the trees following the Johansson scheme (Johansson 1974). Because trees at this locality are relatively small, we defined only four instead of the typical five epiphytic zones, namely: zone 1: base of the trunk at 0–0.50 m; zone 2: trunk at 0.50 m to approximately 2 m; zone 3: main branches 2–3 m; and zone 4: canopy branches at 3–4 m. Within these plots, we estimated the number of mature and juvenile individuals of each species for each zone and collected a voucher and a silica sample for each species; specimens were deposited at the herbaria HUTPL (Loja, Ecuador), VT (USA), and Z/ZH (Switzerland). To test whether different tree zones differ in the assemblage composition and abundance of grammitid species, we performed a principal component analysis (PCA). In order to learn more about *Ascogrammitis lehnertii* and *Mycopteris leucosticta*, the focal species of the present paper, we further used

contingency tables to specifically test for differences in the number of fertile and sterile individuals between zones in these species.

Molecular Phylogenetic Analyses—The systematic position of *A. lehnertii* was determined by inclusion of 14 other species of *Ascogrammitis* for which sequence data was available (Labiak et al. 2010; Sundue et al. 2010, 2014). As outgroups, we included five species of *Mycopteris*, the sister genus, and one species of *Galactodenia*, the next closest genus (Sundue et al. 2010, 2014). We PCR-amplified five plastid DNA markers from the holotype: the *atpß* and *rbcL* coding regions, along with the *rps4-trnS*, *trnG-trnR*, and the *trnL-trnF* intergenic spacers. DNA extraction and PCR amplification protocols followed those of Labiak et al. (2010). DNA sequencing was performed by Genewiz, Boston, Massachusetts. Sequences generated as part of this study were submitted to GenBank. Accession numbers and vouchers for all sequences used in our analyses are provided in Appendix 1. The aligned data matrix is available via Dryad (Sundue et al. 2018).

Sequences were edited and contigs were produced using Geneious 6.17 (Biomatters Ltd., San Francisco, California) and the MAFFT plug-in was used to produce alignments (Katoh and Standley 2013). For each aligned marker, optimal data partitioning and models of substitution evolution were estimated using AICc in PartitionFinder 2 (Lanfear et al. 2012, 2016), which uses PhyML (Guindon et al. 2010). The resulting best scheme included three partitions with separate GTR 1 G models, *atpß* and *rbcL*, *rps4-trnS*, and *trnG-trnR* and *trnL-trnF*. These were implemented in likelihood tree searches using RAxML (Stamatakis 2006) through the CIPRES portal (Miller et al. 2010) with independent searches for the 'best tree' and with 1000 bootstrap replicates.

Taxonomic Treatment

Ascogrammitis lehnertii Sundue, *sp. nov.* TYPE: ECUADOR. Prov. Loja: Podocarpus National Park, Cerro Toledo Area, -4.384526, -79.110965, forest along the road, 3200 m, 3 Jul 2014, *I. Olivares et al. 158* (holotype: VT!, isotypes: HUTPL!, Z/ZH!).

Diagnosis—The new species differs from all other *Ascogrammitis* by up to 2 mm long setae present among the sporangia.

Epiphytes. Roots proliferous and forming new rhizomes. Rhizomes 2–3 mm wide, rhizome scales 3.5–5 3 0.5 mm, lanceolate, clathrate, the cell walls dark brown to blackish, the lumina clear but narrow, and thus the scales appearing blackish in mass, the margin entire or with occasional marginal cilia, the cilia 0.1 mm long, dark brown to blackish, the base rounded, subcordate, the apex attenuate, with a minute subapical glandular cell. Leaves 10–25 3 (2–)3–4 cm, provided with the hyphae and black clavate ascomes of *Acrospermum*, the ascomes 1 mm long; petioles 3–6 cm long, blackish, moderately setose, the setae 1–2 mm long, reddish, spreading; rachis blackish, moderately setose, the setae 1.5–2.0 mm long, reddish, spreading; laminae narrowly elliptic, widest in the middle, 1-pinnatisect, the base attenuate, with 5–10 pairs of gradually reduced pinnae, the apex acute, pinnatifid; pinnae oblong, medial pinnae 1–2 3 0.2–0.35 cm, the apices acute, the bases slightly expanded, proximal pinnae gradually reduced to shallow lobes 0.5 3 3 mm, distal pinnae gradually reduced; pinna costae blackish, visible on both sides of the lamina; veins not darkly colored and not clearly visible; abaxial lamina surface provided with scattered simple 2-celled trichomidia, also moderately setose, the setae 1.5–2.0 mm long, reddish, spreading, concentrated primarily along the pinna costae and within sori; adaxial lamina surface with occasional 0.2–0.5 mm long setae along the pinna costae, otherwise glabrous; hydathodes conspicuous, noncretaceous, or if so then the deposit fallen off of the material seen. Sori medial, slightly elongate. Spores green, normally developed. Figure 1.

Distribution and Ecology—These plants are low-canopy epiphytes forming small colonies from proliferous roots.

The rarity of *A. lehnertii* is difficult to determine. Despite previous intensive collection efforts in Loja for the past 30 yr (Kessler and Lehnert 2009a, b; Mandl et al. 2010), all but one of the collections of this new species were made in the last three years. It is common on Cerro Toledo, with over 2000 individuals recorded on 6400 m^2, but is known only from two other locations. The discovery of this new species highlights the patchy distribution of some species in the Andes and the value of surveying habitats that potentially host unrecognized plant diversity.

Etymology—The new species is named in honor of Marcus Lehnert in recognition of his numerous contributions to fern systematics and taxonomy. Marcus conducted extensive fieldwork near the type locality of the new species, leading to the checklist of Pteridophytes to Reserva Biológica San Francisco, Ecuador (Lehnert et al. 2007).

Notes—*Ascogrammitis lehnertii* is distinguished from its congeners by the combination of its relatively broad lamina, (2–)3–4 cm wide, fertile leaves bearing 2 mm long reddish setae in and near the sori, and by bearing proliferous roots from which new plants emerge. Only two other species of *Ascogrammitis* have proliferous roots, *A. anfractuosa* (Kunze ex Klotzsch) Sundue and *A. alan-smithii* (A. Rojas) Sundue. These are easily distinguished by their narrower laminae, (0.4)0.8–1.4 cm wide, and small ovate rhizome scales 0.5–0.7 3 0.2–0.3 mm (vs. 3.5–5 3 0.5 mm in *A. lehnertii*). Proliferous roots can be detected on specimens by looking for cases where multiple rhizomes have developed from a single root system. If the proliferous roots are overlooked on specimens, *Ascogrammitis lehnertii* is likely to be confused with A. *loxensis* Sundue and *A. dilatata* Sundue, which share similar laminae, and have non-setose pinna margins. From these, *Ascogrammitis lehnertii* can be distinguished by the up to 2 mm long reddish pluricellular setae emerging from the sori. These are in fact the longest abaxial setae of any *Ascogrammitis* and should distinguish *A. lehnertii* from all other species in the genus.

Additional Specimens Examined—Ecuador.—PROV. LOJA: Podocarpus National Park, Cerro Toledo Area, -4.384526°, -79.110965°, forest along the road, 3200m, 3 Jul 2014, *I. Olivares et al. 142* (HUTPL, VT, Z); idem *I. Olivares et al. 143* (HUTPL, VT, Z); idem *I. Olivares et al. 160* (VT, Z).—PROV. ZAMORA-CHINCHIPE: Podocarpus National Park, Bombuscaro area, forest 5 m from the walking trail towards "El Mirador", -4.112886°, -78.968189°, 1200 m, 26 Jun 2014, *I. Olivares et al. 48* (HUTPL, VT, Z); Road Loma del Oro (S of Saraguro) toward Fierro Urcu, ca. km 9, 3400 m, [-3.95°, -79.583°], 12 Mar 1989, *B. Øllgaard, J. Madsen and L. Ellemann 91041* (AAU).

Results

Phylogenetic Analysis—Analysis of cpDNA (Fig. 2) resolved *Ascogrammitis* as monophyletic with strong bootstrap support (BS 100%). The overall topology was similar to that of previous studies (Sundue et al. 2010, 2014), but differed in some respects. In our results, *Ascogrammitis nana* is resolved as sister to *A. anfractuosa* in the first divergent clade, whereas in previous analyses it was resolved as sister to *A. pichinchense*, the second divergent lineage. Similar to previous analyses, the remaining species formed a well-supported clade (BS 100%), but with some weakly supported in- ternal nodes that inhibit detailed interpretation of the relationships.

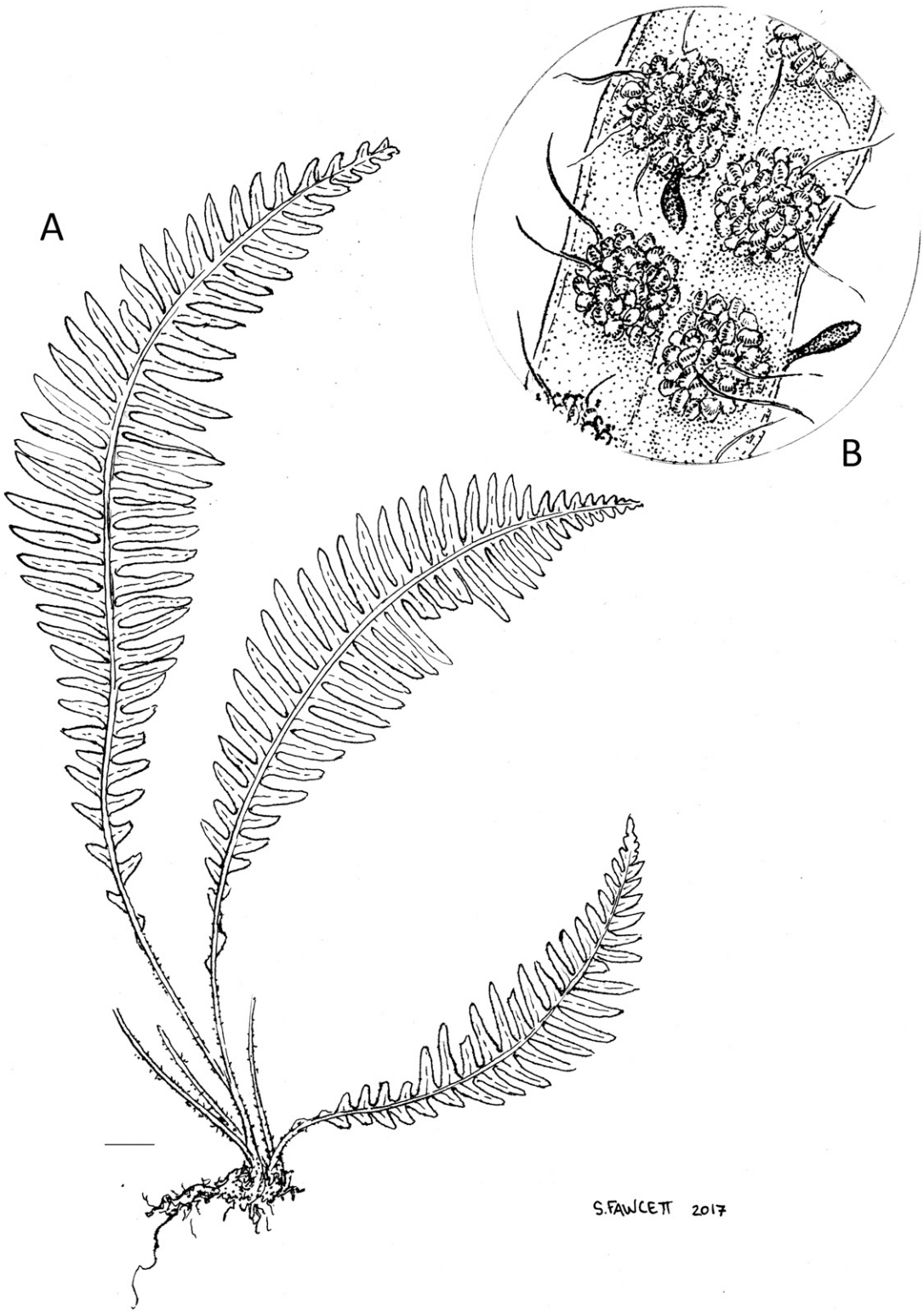

Fig. 1. *Ascogrammitis lehnertii* A. Habit; scale bar 5 1 cm. B. Detail adaxial lamina (*Olivares et al. 142,* VT).

Phylogenetic Relations of Ascogrammitis lehnertii—The new species was resolved with strong support (BS 100%) as sister to *A. cuencana* (Fig. 2), an infrequently collected species known from the Western cordillera of Ecuador and the Central cordillera of Colombia (Sundue 2010). These species together were supported as sister to *A. dilatata*, a species known from Bolivia and southern Peru (Sundue and Kessler 2008; Sundue 2011).

Grammitid Community Assembly—At the type locality on Cerro Toledo, we recorded a total of 7986 individuals of 16 grammitid species in our eight study plots of 400 m^2 each. The most abundant species was *Stenogrammitis jamesonii* (Hook.) Labiak (2472 individuals, 31%) followed by *Ascogrammitis lehnertii* (2069 individuals, 26%), and *Mycopteris leucosticta* (818 individuals, 10%). Other five species also had over 100

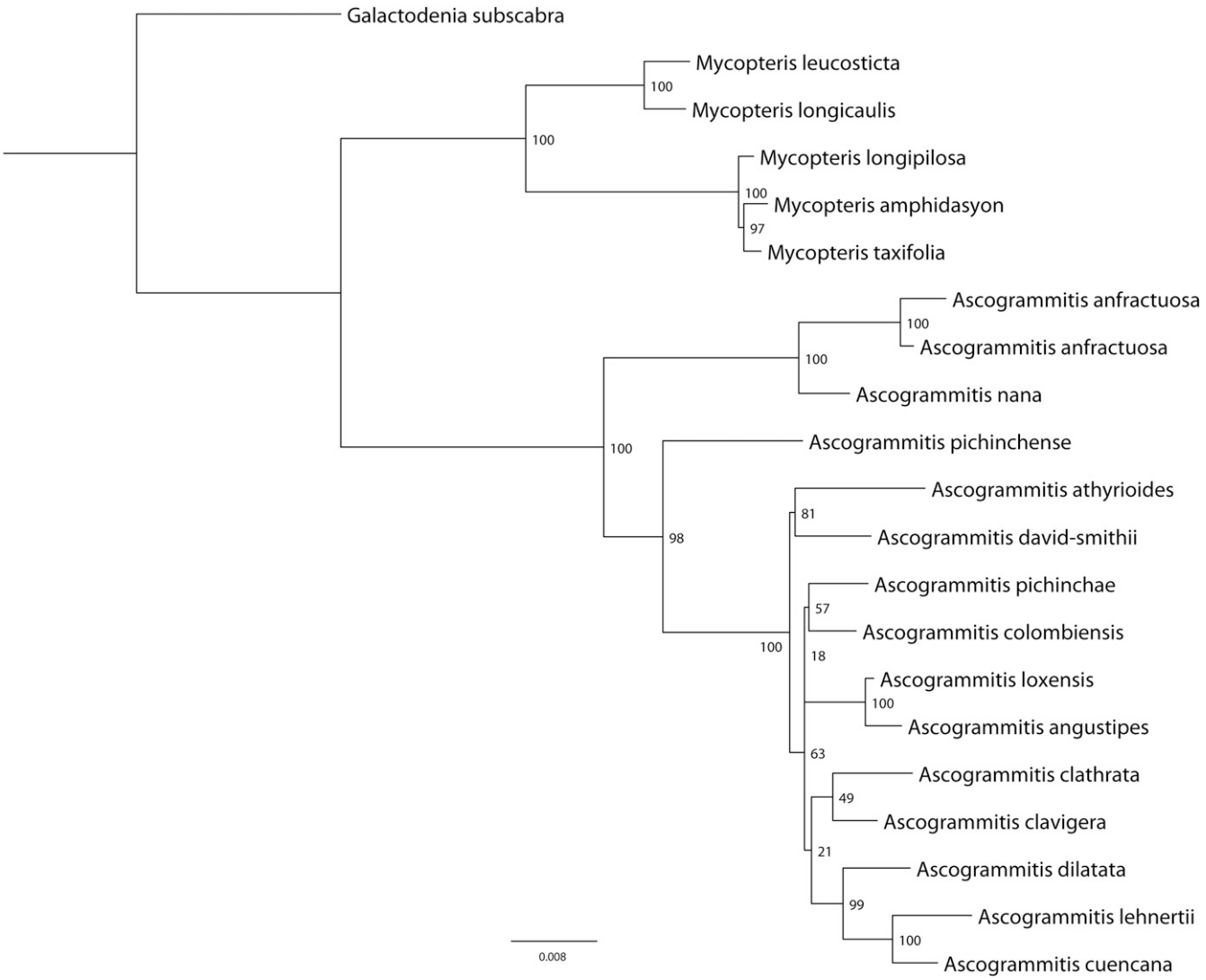

Fig. 2. Best tree resulting from the maximum likelihood analysis of the five chloroplast markers (*atpB*, *rbcL*, *rps4*, *trnL-trnF*, *trnG-trnR*). Numbers at nodes are bootstrap support values. Scale bar represents substitutions per site.

individuals each. The PCA showed that the vertical distribution of grammitid species was not random (Fig. 3). About 95% of the variance in the distribution of species was explained by the frequency of species at either the bases of the trees (z1) or the trunks (z2), and only 5% of the variance was explained by the frequency of some species on the main branches (z3). Only a few individuals of the two most common species were present on the canopy branches (z4), therefore this zone does not explain any of the variance. Differences in the vertical distribution of individuals were significant for the eight most abundant species ($x^2 = 974.61$, df = 21, $p < 2.2^{e-16}$).

The relative distribution of fertile (mature) and sterile (juvenile) individuals also varied within all most common species in the community (Fig. 4). Specifically, for the previously unknown *A. lehnertii* (Fig. 4b) 47% of all individuals in zone 1 were fertile, whereas in zones 2–4 only 35% were fertile ($x^2 = 34.26$, df = 3, $p = 1.74^{e-07}$). In *Mycopteris leucosticta* (Fig. 4c), 52% of all individuals in zones 1–2 were fertile, whereas in zone 3 only 36% were fertile ($x^2 = 9.44$, df = 2, $p = 0.008$).

We also recorded three individuals apparently of *A. lehnertii* at Bombuscaro, a locality at lower elevation (1200 m), however, these were sterile and we cannot confirm their identity with certainty.

Discussion

Ascogrammitis belongs to the Polypodiaceae subfamily Grammitidoideae (PPGI 2016) as evident by the round exindusiate sori, reddish pluricellular setae, and chlorophyllous trilete spores (Sundue et al. 2010). They are predominantly epiphytic species of Neotropical cloud forests, known primarily from the northern and central Andes, with one species ranging into Central America and the West Indies (Sundue 2010). The genus is diagnosed by having dorsiventral rhizomes with ventral root insertion and clathrate rhizome scales with cordate bases and reddish setose margins. The fronds bear numerous reddish setae, and hydathodes are present and often cretaceous (Sundue et al. 2010). The name *Ascogrammitis* is derived from the relationship of its species with *Acrospermum*, an epibiotic ascomycete that grows upon the leaves of these ferns. These fungi also occur on the grammitid genus *Mycopteris* Sundue, and a few other species of Polypodiaceae (Sundue 2010).

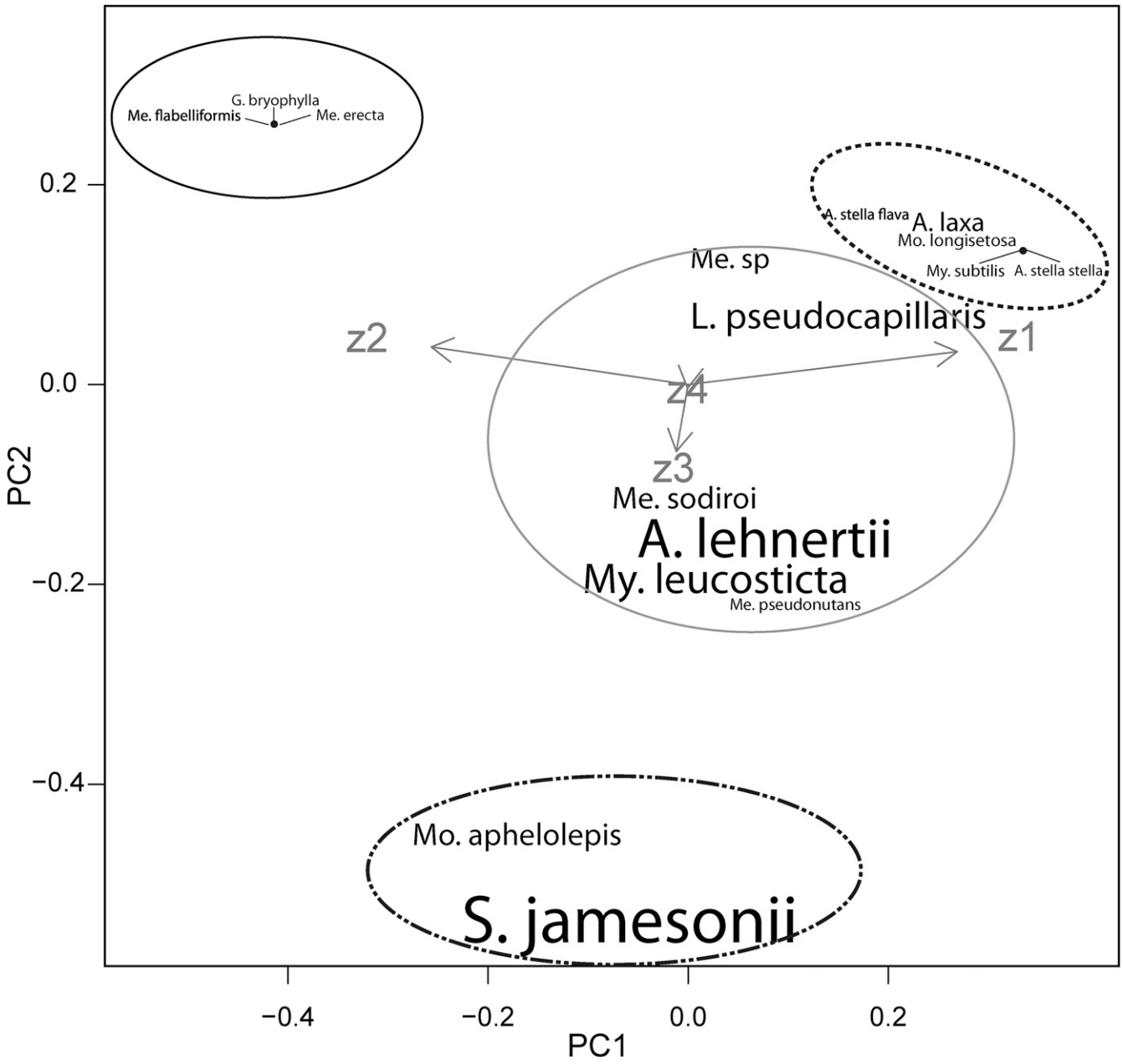

Fig. 3. Principal component analysis (PCA) for the relative abundance of species present in four Johansson epiphytic-zones (z1–z4) at Cerro Toledo. Species were distributed in four groups, one group (black dotted ellipse) preferred the bases of the trees (Johansson zone 1, z1), a second group (solid black ellipse) contained species that were only present on the trunk (z2), the third group did not show a clear preference between the trunks and main branches (z1–z3, grey solid ellipse), and group four included species with relative high abundances on the main branches (z3, dashed-dotted ellipse). The size of species names indicates the relative abundance of each species. Full species names: *Alansmia laxa, A. stella* var. *flava, A. stella* var. *stella, Ascogrammitis lehnertii, Grammitis bryophila, Lellingeria pseudocapillaris, Melpomene erecta, M. flabelliformis, M. pseudonutans, M. sodiroi, M.* cf. *personata, Moranopteris aphelolepis, M. longisetosa, Mycopteris leucosticta, M. subtilis, Stenogrammitis jamesonii*.

With the addition of this new species, *Ascogrammitis* now comprises 18 species. With the addition of *A. lehnertii*, Ecuador is now home to six species in the genus, the others being *A. anfractuosa*, *A. loxensis*, *A. pichinchae*, *A. pichinchensis*, and *A. tungurahuae*. With *A. lehnertii*, *A. pichinchensis*, and *A. tungurahuae* being endemic to the country, Ecuador can be said to have the highest number of endemic species of *Ascogrammitis* compared to other countries.

Grammitid Community Assembly—The grammitid community studied here contained 16 species from eight genera, with an average of 5.8 species per plot of 400 m². This diversity is equivalent to that found elsewhere in the tropical Andes and Costa Rica at these elevations (Kessler 2001; Kluge et al. 2006; Salazar et al. 2015) as well as in Southeast Asia and New Guinea (Kessler et al. 2001; M. Kessler et al. unpubl. data). The most species-rich genus was *Melpomene* A. R. Sm. & R. C. Moran with five species. This genus is typical for high elevation forests in the Neotropics and indeed includes the highest-growing vascular epiphytes worldwide (Sylvester et al. 2014). Compared to extensive data of methodologically similar plots elsewhere in the Andes and Central America (e.g. Kessler 2001; Kluge et al. 2006; Salazar et al. 2015), the

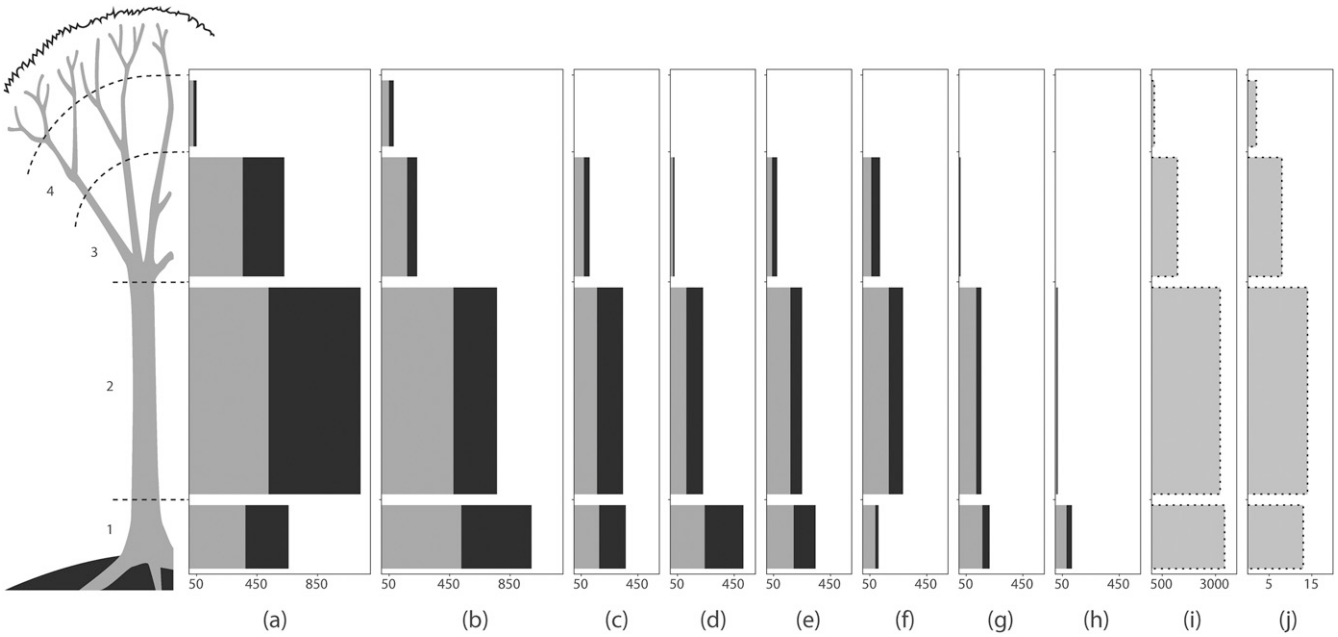

Fig. 4. Vertical distribution of the eight most abundant grammitid species on Cerro Toledo following the vertical zonation of Johansson with zone 1 corresponding to the trunk bases, zone 2 to the trunks, zone 3 to the main branches, and zone 4 to the canopy branches. Bars indicate the number of sterile (gray) and fertile (black) individuals per zone. In the two rightmost panels we show the total numbers of individuals (i) and species (j) per zone. (a) *Stenogrammitis jamesonii*, (b) *Ascogrammitis lehnertii,* (c) *Mycopteris leucosticta*, (d) *Lellingeria pseudocapillaris*, (e) *Melpomene sodiroi*, (f) *Moranopteris apheloleps*, (g*) Melpomene* cf. *personata*, (h) *Alansmia laxa*.

grammitid community at Cerro Toledo is fairly typical in terms of species richness and abundance of individuals.

Ascogrammitis lehnertii was the second most abundant species at Cerro Toledo after *Stenogrammitis jamesonii*. These two species showed clear habitat segregation: *A. lehnertii* was significantly more abundant at the trunk bases whereas *S. jamesonii*, although present from the bases of the trunks to the canopy, showed a clear preference for the branches. Other species of different genera shared these two main microhabitat preferences, with *Lellingeria pseudocapillaris* (Rosenst.) A.R. Sm. & R.C. Moran sharing the trunk bases with *A. lehnertii*, and *Moranopteris apheloleps* (C.V. Morton) R.Y. Hirai & J. Prado preferring higher strata, like *S. jamesonii*. These tendencies were even more pronounced when considering only mature, fertile individuals. In *A. lehnertii*, for example, these were most abundant at the trunk bases, forming a "source population" whereas mostly sterile individuals were present in the higher zones and conformed "sink populations." Previous studies have shown that microclimatic conditions differ among the epiphyte zones and that they correlate with the distribution of epiphytes (Cardelús and Chazdon 2005; Zotz 2016). Compared to the other zones, the base of the trunk is the most humid and, at this elevation, least cold of all microhabitats (Krömer et al. 2007). Hence, it seems that *A. lehnertii* tends to occupy the less extreme part of the microenvironmental gradient in the forest patches of Cerro Toledo, although light is presumably most limited here. Clearly, different species of grammitid ferns show different microhabitat preferences, but to which degree these are driven by the ecophysiologically determined adaptations of the species or by competitive interactions between species (Karger et al. 2015) remains to be studied in detail. In any case, species coexistence in these diverse epiphytic communities is linked to habitat segregation.

The new record of *Mycopteris leucosticta,* a species previously restricted to the Chocó of Ecuador, confirms that migration of the species occurs across the Amotape-Huancabamba zone. Several studies have demonstrated the important role of this area, which constitutes a corridor for the dispersal of mountain and lowland species in both directions North-South and West-East (Weigend 2002).

Key to the Species of *Ascogrammitis*

Note: *A. dilatata* and *A. loxensis* are keyed twice to accommodate variation.

1. Roots proliferous, plants forming small to large colonies. 2
   2. Rhizome scales lanceolate, 3.5–5 mm long; leaves 10–25 × (2–)3–4 cm. . . . . *A. lehnertii*
   2. Rhizome scales ovate, 0.5 mm long; leaves 5–10 × 0.9–1.4 cm. 3
      3. Hydathodes cretaceous; segment margins setose; adaxial lamina surface with scattered setae; rhizome scale margin entire; plants apparently lacking the mycelia and ascomes of *Acrospermum*; epiphytic, 750–1200 m (Mesoamerica). . . . . *A. alan-smithii*
      3. Hydathodes cretaceous or not; segment margins setose or not; adaxial lamina surface glabrous or with scattered setae; rhizome scale margins entire or ciliate; plants with mycelia and ascomes of *Acrospermum*; epiphytic or epipetric, 1000–3000 m (widespread). . . . . *A. anfractuosa*
1. Roots not proliferous, plants individual. 4
   4. Segments deeply and regularly pinnatifid (Peru, Bolivia). . . . . *A. athyrioides*
   4. Segments entire or minutely denticulate, but not distinct (central and northern Andes) 5
      5. Segment margins and apices evenly setose. 6

6. Laminae membranaceous; fronds pendent; segment apices rounded; dark sclerenchyma of segment costae usually not visible abaxially (Ecuador). . . . . *A. pichinchensis*
6. Laminae chartaceous; fronds erect, arching, or pendent; segment apices acute; dark sclerenchyma of segment costae visible abaxially (central and northern Andes). 7
7. Petiole setae 1–2.5 mm long; setae of segment margins 0.5–1 mm long; sori distributed evenly throughout the frond; segment bases neither conspicuously decurrent nor sucurrent (Colombia, Ecuador). . . . . *A. pichinchae*
7. Petiole setae 0.5–1.5 mm long; setae of segment margins 0.5 mm long; sori confined to the distal portions of the lamina; segment bases decurrent and sucurrent, the proximal ones often conspicuously dilated. 8
8. Lamina base short attenuate, with 4–8 pairs of reduced segments; rhizome scales 1.5–3 × 0.2–0.3 mm; petioles sparsely setose; segment margins irregularly setose; lamina green (central Peru, Bolivia). . . . . *A. dilatata*
8. Lamina base long attenuate, with 16–20 pairs of reduced segments; rhizome scales 2.3–4.2 × 0.3–0.6 mm; petioles moderately setose; segment margins regularly setose; lamina bluish-green (Ecuador, N Peru). . . . . *A. loxensis*

5. Segment margins glabrous or provided with minute hairs, but not setose, segment apices glabrous or provided with 1–3 setae in *A. colombiensis*, *A. cuencana*, *A. david-smithii*, *A. nana, A. stuebelii*. 9
9. Petiole setae 0.2–0.4 mm long; surface of rhizome scales ciliate or glabrous. 10
10. Rhizome scales ciliate on surfaces as well as margins; fronds; 19–38 × 2–4.6(–6), (N Peru). . . . . *A. oxapampensis*
10. Rhizome scales glabrous on surfaces, ciliate on margins; fronds 8–13 × 1.4–2 cm (Colombia) . . . . . *A. stuebelii*
9. Petiole setae 1–2.5 mm long; surface of rhizome scales glabrous, the margin ciliate. 11
11. Laminae 5–7.5 cm wide; 14–22 sori per segment; rhizome scales 4.5 mm long; abaxial lamina surface moderately to densely and evenly provided with erect setae, the sterile and fertile portions of the abaxial lamina equally setose (Bolivia). . . . . *A. clathrata*
11. Laminae up to 4.5 cm wide; 3–14 sori per segment; rhizome scales 3–3.5 mm long; abaxial lamina nearly glabrous to densely setose, the fertile portions of the abaxial lamina generally more densely setose than sterile portions. 12
12. Hydathodes non-cretaceous; rhizomes bearing branch buds; petiole bases with two vascular bundles (check the portion of the petiole immediately adjacent to the rhizome). 13
13. Abaxial lamina moderately setose, the setae 0.5–1 mm long; rhizome scales 2.5–3.5 mm long (Colombia). . . . *A. colombiensis*
13. Abaxial lamina glabrous or with scattered setae 0.5 mm long; rhizome scales 4–5 mm long (Venezuela). . . . . *A. clavigera*
12. Hydathodes cretaceous (the whitish deposit sometimes lost); rhizomes lacking branch buds; petioles with a single vascular bundle (check the portion of the petiole immediately adjacent to the rhizome). 14
14. Fronds 14–20 × 0.8–2.8 cm, erect. 15
15. Rhizome scales 0.2–0.3 mm wide; petiole setae 1.5–2 mm long; laminae 1.2–2.8 cm wide (Peru, Bolivia). . . . . *A. nana*
15. Rhizome scales 0.7 mm wide; petiole setae 0.5–1 mm long; laminae 0.8–1.6 cm wide (Colombia, Ecuador). . . . . *A. cuencana*
14. Fronds 18–60 × 2–4.5 cm, arching or pendent. 16
16. Laminae deeply 1-pinnatifid to 1-pinnatisect, the tissue between segments sometimes very narrow, but always connected. 17
17. Receptacular setae absent; sori 4–8 per segment, evenly distributed throughout the lamina; pinna costae not readily visible abaxially (Colombia) . . . . . *A. angustipes*
17. Receptacular setae present; sori up to 12 per segment, usually confined to the distal portion of the lamina; pinna costae visible abaxially 18
18. Lamina base short attenuate, with 4–8 pairs of reduced segments; rhizome scales 1.5–3 × 0.2–0.3 mm; petioles sparsely setose; segment margins irregularly setose; lamina green (central Peru, Bolivia). . . . . *A. dilatata*
18. Lamina base long attenuate, with 16–20 pairs of reduced segments; rhizome scales 2.3–4.2 × 0.3–0.6 mm; petioles moderately setose; segment margins regularly setose; lamina bluish-green (Ecuador, N Peru). . . . . *A. loxensis*
16. Laminae 1-pinnate nearly throughout. 19
19. Rhizome scales with narrow indistinct lumina, blackish in mass; fronds 27–60 cm long (Ecuador). . . . *A. tungurahuae*
19. Rhizome scales with broad distinct lumina, dark grey in mass; fronds 18–37 cm long (Bolivia). . . . *A. david-smithii*

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Author Contributions

The morphological diagnosis and phylogenetic analyses were conducted by MS. Field work and the analysis of community assembly were conducted by IO. All three authors conceived and contributed to writing the manuscript.

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Appendix 1. Genbank accession numbers for DNA sequences used in this study. Information is presented in the following order: taxon, voucher, *atpB*, *rbcL*, *rps4-trnS*, *trnG-trnR*, *trnL-trnF*. Sequences generated as part of this study are in bold face.

Ascogrammitis anfractuosa (*Lehnert 1035*) GU476783, GU476853, KM106108, KM105967, GU476675. *Ascogrammitis anfractuosa* (*Kessler 14867*) MG948936, MG948938, MG966515, MG966517, MG966519. *Ascogrammitis angustipes* (*Sundue 1237*) KM218837, GU476891, KM106109, KM105968, GU476703. *Ascogrammitis athyrioides* (*Lehnert 261*) KM218840, GU476856, KM106110, KM105969, GU476704. *Ascogrammitis clathrata* (*Kromer 1237*) KM218838, GU476843, KM106111, KM105970, GU476708. *Ascogrammitis clavigera* (*Schneider 2400*) KM218839, GU476925, KM106112, KM105971, GU476709. *Ascogrammitis colombiensis* (*Sundue 1316*) GU476804, GU476900, KM106113, N/A, GU476710. *Ascogrammitis cuencana* (*Lehnert 1164*) N/A, GU476851, KM106114, GU387205, GU476714. *Ascogrammitis* david-*smithii* (*Sundue 785*) GU476794, GU387012, GU387122, N/A, GU476688. *Ascogrammitis dilatata* (*Labiak 4728*) GU376640, GU387033, GU387124, GU387206, GU387285. *Ascogrammitis lehnertii* (*Olivares 142*) MG948937, MG948939, MG966516, MG966518, MG966520. *Ascogrammitis loxensis* (*Sundue 1164*) GU476812, GU386995, GU387125, GU387207, GU476721. *Ascogrammitis nana* (*Labiak 4725*) GU376642, GU387031, GU387126, GU387208, GU387287. *Ascogrammitis pichinchae* (*Wilson 2816a*) AY459508, GU476928, KM106115, N/A, GU476730. *Ascogrammitis pichinchense* (*Lehnert 1577*) GU476816, GU476854, KM106116, N/A, GU476732. *Galactodenia subscabra* (*Moran 8078*) GU476821, GU476860, GU387127, GU387209, GU476739. *Mycopteris amphidasyon* (*Moran 7646*) GU476759, GU476922, KM106161, KM106007, GU476638. *Mycopteris leucosticton* (*Lehnert 1128*) GU476811, GU476848, KM106162, N/A, GU476720. *Mycopteris longicaulis* (*Jimenez 373*) GU476813, GU476840, KM106163, N/A, GU476724. *Mycopteris longipilosa* (*Sundue 1033*) GU476814, GU476861, KM106164, KM106008, GU476726. *Mycopteris taxifolia* (*Labiak 4018*) GU476800, GU476914, KM106167, KM106009, GU476699.

Chapter 4

Are epiphytic ferns reaching saturation?

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Abstract:
A recent review on the concept of species saturation defined it as *the dynamic equilibrium of species richness due to niche limitation within a given group of organisms and within an environmental and temporal reference frame.* Thus, to demonstrate saturation, we need evidence for both spatial or temporal stability or equilibrium of species numbers and for niche limitation. Epiphytic plants are an interesting group to test for species saturation because they are the second most diverse group of epiphytes after orchids. However, it is commonly believed that competition only plays a minor role in epiphytic plant communities because they often grow at low densities and because of their highly unstable habitat. In this study, we explored the possibility of local species saturation within grammitid ferns. To do this we analysed the composition of grammitid communities in 1090 forest-plots from America, Africa, and Asia by (a) testing if there are differences in the distribution of grammitid species richness along the temperature and precipitation gradients in these three independent regions and, by (b) studying the relationship between the local numbers of species and individuals per species found in each plot in America and Asia. We conclude that there is preliminary evidence indicating that the number of species of grammitid ferns that co-occur at a local plot scale is at or approaching the level of ecological saturation. This is in contrast with the evidence at the regional scale, which shows no evidence of a slowdown in diversification rates. Our results are in line with ecological and evolutionary theory, which predicts that while there is a strong feedback between local and regional dynamics different mechanisms operate at each scale and therefore their biodiversity patterns must not necessarily be synchronised.

Introduction
Is there a maximum number of species that can coexist in a given assemblage? Or that a lineage can produce over time? In theory, it is obvious that in a world of limited space and resources, these limits must exist (Schluter 2000, Ricklefs 2006, Phillimore and Price 2008, Rabosky and Hurlbert 2015, Gascuel et al. 2015), but defining where precisely they lie and demonstrating their causes has proven problematic. After Grinnell (1914) and Elton (1950) published their initial ideas about species richness being limited by niche space, MacArthur and Wilson (1967) proposed that this “saturated” state was the result of an equilibrium between the rates of immigration and emigration of species. During the decades that followed these pioneer studies, numerous studies have attempted to test whether ecological communities have reached saturation level or not. As a result, many studies support the idea of saturation (e.g., Terborgh and Faarborg 1980, Sepkoski 1984, Alroy 2008, Rabosky and Lovette 2008, Rabosky and Hurlbert 2015) whereas many others reject it (e.g., Benton 1995, Cornell 1999, Stohlgren et al. 2008, Harmon and Harrison 2015). Much of this uncertainty does not only originate from the ecological complexities as such, but rather from theoretical misconceptions (Loreau 2000), lack of a clear analytical framework (Cornell and Lawton 1992, Cornell 1999, Russell et al. 2006), and lack of robust methodological approaches(Palmer and van der Maarel 1995, Cresswell et al. 1995, Mateo et al. 2017).

For a long time, the lack of a consistent terminology has hampered the differentiation between the patterns resulting from saturation from the processes and mechanisms that cause it. In this regard, a recent review on this concept defined saturation as *the dynamic equilibrium of species richness due to niche limitation within a given group of organisms and within an environmental and temporal reference frame* (Olivares et al., *in press*). Thus, to demonstrate saturation, we need evidence for both spatial or temporal stability or equilibrium of species numbers and for niche limitation. It thus must be considered that equilibrium in the context of saturation refers to the dynamic process whereby there is a balance of species additions (via immigration or speciation) and losses (via emigration or extinction) in a given system. As a result of this dynamic equilibrium, we observe stability in the number of species.

Thus, stability and equilibrium constitute indicators that saturation might occur. However, it is only possible to determine if saturation occurs when there is also an indication that stability or equilibrium are reached by means of niche limitation and not through stochastic mechanisms. The underlying assumption stems from Gause's principle, based on the Lotka-Volterra competition model, according to which species can stably coexist through the exploitation of different niches(Gause 1934, Silvertown 2004). Niche limitation mechanisms originate from a wide range of ecological and evolutionary reasons, including, e.g., minimum population sizes (Cornell 1999), limits to the similarity of species (MacArthur and Levins 1967, Chesson 2000a), species interactions (Shurin and Allen 2001, Amarasekare 2003, Stachowicz and Tilman 2004), and niche characteristics set by the environment (Wiens 1974, Chesson 2000b, Jetz and Fine 2012). Accordingly, a full assessment of saturation requires an assessment of some of these mechanisms.

In the light of niche limitation and competition principles (Hutchinson 1957), epiphytic plants are an intriguing group because it is commonly believed that competition only plays a minor role in epiphytic plant communities (Zotz 2016). The rationale behind this is that most epiphytes (except mosses) often grow at such low densities that they are rarely in contact with one another. In addition, environmental conditions (wind, temperature, light, and humidity) are very dynamic in the epiphytic habitat and branches are continuously growing and decaying. This instability might hamper the expansion of epiphytes to achieve densities at which space, light, water, or nutrients become limiting, preventing species to experience much competitive pressure (Benzing 1990). In fact, Hutchinson (1957) describes highly unstable habitats as one of the cases where the "Volterra-Gause principle is unlikely to apply" because constant environmental dynamics would continually change the direction of competition preventing the elimination of one competitor.

Epiphytic ferns are an interesting group to test hypotheses on saturation because with some 2700 species, they are the second most diverse group of epiphytes after orchids (Zotz 2013), and through their evolutionary history underwent radiation events in many areas of the world (Sundue et al. 2015). Having such high diversity across the globe, the question arises if it were possible that at some level the diversification of epiphytic ferns has been limited and they might have even reached saturation under certain conditions.

Polypodiaceae is the most species rich family of epiphytic ferns with *ca*. 1250 epiphytic species (Zotz 2013) and has the highest diversification rate of all epiphytic fern families (Testo and Sundue 2016). Two-thirds of the diversity in Polypodiaceae belongs to the grammitid ferns, a monophyletic clade that contains approximately 900, mostly epiphytic species. Recent phylogenetic studies indicate that grammitids originated in the Neotropics at the end of the Eocene (37–44.9 Ma) (Sundue et al. 2014). Long-distance dispersal events of spores across continents are responsible for the colonization of grammitid ferns in Asia and Africa. A single colonisation event from America to Asia at around 22.4-27.3 Ma led to an Asian radiation that contains the greatest diversity of grammitids with c. 490 species (Sundue et al. 2014). In contrast, at least 13 colonisation events have occurred from America to Africa but none of them has resulted in a major radiation. In fact, Africa is the least diverse of the three regions (Table 1) and most of the African species are restricted to Madagascar (Bauret et al. 2017).

This biogeographical situation creates a highly suitable opportunity to test for ecological saturation because the three continental regions have markedly different species numbers and diversification rates (about twice as high in Asia than in America; very low in Africa). Globally, phylogenetic studies suggest that there has been no slowdown in the diversification rate of grammitid ferns (Testo and Sundue 2016), suggesting that regional diversity is not saturated. However, a study comparing species composition of two diverse local assemblages in the Bolivian Andes and on Mt. Kinabalu (Borneo) found significant convergence in the number of species and growth forms between the two areas, despite largely independent evolutionary histories of their floras and different regional richness levels (Kessler et al. 2001). These results are indicative of selective pressure with certain habitats offering a limited number of niches and favouring particular growth forms, which might be the result of saturation at the local level. It would thus be possible that local assemblages do not directly depend on the clade's regional diversity and are governed by local constraints where other factors (mostly environment) are responsible for local diversity patterns. However, the study of Kessler et al.

(2001) was based on less than 100 vegetation plots from only two sites, putting into question the generality of their observations, and no specific tests were made for saturation.

Table 1. Biogeographical features of grammitid ferns (Polypodiaceae) on the three major tropical continental areas. Historically, the diversification of Polypodiaceae has been attributed to two phytogeographic zones i) Neotropics-Africa-African Islands (Madagascar, Mascarenes, Seychelles and Comoros) and ii) Asia-Malesia-Pacific. In this paper, we study the grammitid flora in the three biogeographic areas of America, Africa, and Asia (-Melanesia).

Biogeographical feature	**America**	**Africa**	**Asia**
First appearance of grammitid ferns	37-45 Ma	14-21 Ma	22-27 Ma
Number of species	350	51	490
Origination/colonisation events	1	at least 13	1
Major radiations	1	0	1
Shared genera	with Africa: 13 with Asia: 0	with America: 13 with Asia: 1	with Africa: 1 with America: 0

In the present study, we set out to explore the possibility of local species saturation within grammitid ferns, by specifically asking if there is any evidence of equilibrium and niche limitation in grammitid assemblages in the three main regions where they occur. To do this we analysed the composition of grammitid communities in forests from America, Africa, and Asia and asked a) if there is evidence of stability and b) if there is evidence of niche limitation. We approached these questions by testing a) if there are differences in the distribution of grammitid species richness along the temperature and precipitation gradients in these three independent regions, and (b) by studying the relationship between the local numbers of species and individuals per species found in each plot in America and Asia. The basic predictions emerging from these tests are that, if communities are saturated, then a) the local numbers of species should not differ between continents despite the large differences in species pools and b) that the number of individuals per species should not increase proportionately with species numbers, indicating that there is a limitation of the number of individuals.

Methods

Plots — We used a dataset encompassing 1090 vegetation plots in seven countries in America (740 plots), four countries in Africa (43 plots), and five countries in Asia (307 plots) (Fig 1). Much of the dataset (840 plots) was assembled during previous studies and is more fully described in (Kluge et al. 2006, Kluge and Kessler 2007) for Costa Rica, (Kessler 2000a, Salazar et al. 2015) for Ecuador, Kessler et al. (2010) for La Réunion, and (Karger et al. 2014) for Asia. Another part of the data was collected since 2014 as part of studies on elevational gradients carried out by all authors. In each country, plots

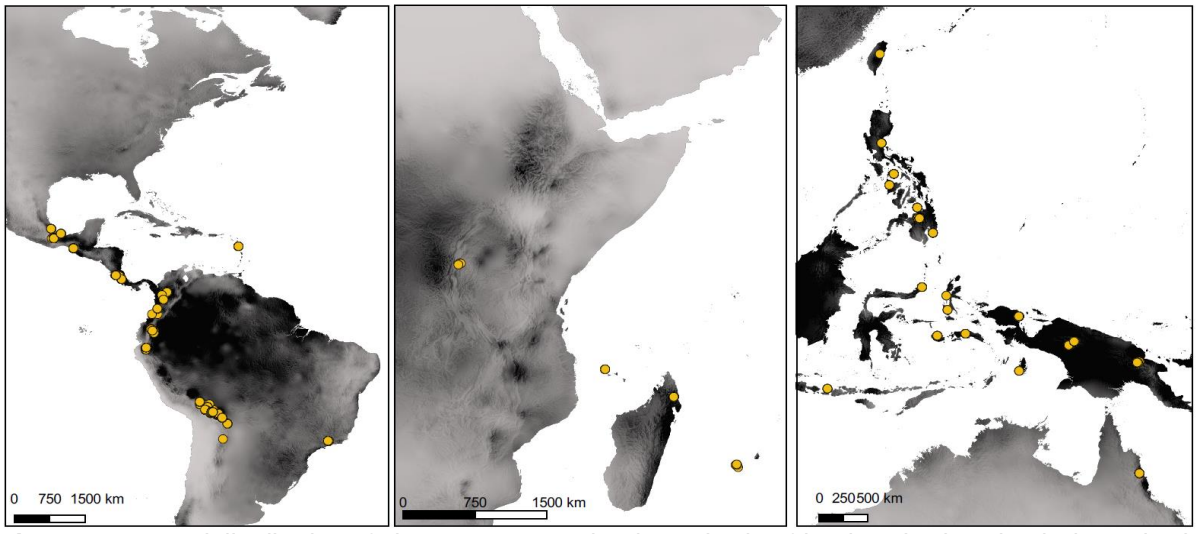

Figure 1. General distribution of plots across America (740 plots), Africa (43 plots) and Asia (307 plots).

were distributed along elevational gradients and were established in homogeneous forest habitats that had no or little evidence of human disturbance. Azonal vegetation (e.g., ravines, secondary vegetation) was not sampled. Each plot was 400 m^2 (usually 20 m x 20 m) in size. In each plot, all grammitid species were identified. In plots established after 2011 (N = 603), we also estimated the numbers of individuals per species. Voucher specimens were collected and deposited in the corresponding national herbaria as well as in the herbaria GOET, UC, and Z.

Climate —The distribution of ferns is highly correlated with precipitation and temperature (Kessler 2000b, Krömer et al. 2005, Kluge et al. 2006) and therefore we only used these variables for characterising the climatic coverage of our sampling. We downloaded data on mean annual precipitation and temperature for each plot from CHELSA-climate.org (Karger et al. 2017).

Analyses

Stability — We used multiple regression models to test if the number of grammitid species found along temperature and precipitation gradients was significantly different between the three continents. Stability would be rejected if different numbers of species are found under a given value of temperature and precipitation in the three regions. The three regions studied differ in the distribution of precipitation and temperature (see results). Therefore, to test for the stability in species richness distribution along the environmental gradient, we included region as a covariate of the precipitation and temperature models. In addition, we tested for differences between all pairs of regions.

Niche limitation — Here we used data from 603 plots (America=296, Asia=307) and omitted the rest because they only included presence/absence data. We defined species richness as the total number of species present in each plot. Similarly, we defined density as the average number of individuals per species present in each plot. We excluded Africa from the individuals-based analyses because we only had 8 plots with grammitid individuals data of a single species from a single site. The sub-dataset included individuals' data for 237 grammitid species.

We tested if there was evidence of niche limitation by assessing the relationship between species richness and density across all plots in America and Asia. We used the Akaike Information Criterion (AIC) to test which of three models (linear, squared, or logarithmic) best described the relationship between richness and density. Evidence for niche limitation would be accepted if the logarithmic model performed better than the rest, indicating that the number of individuals is limited.

Results

We collected data for a total of 280 species, corresponding to *ca.* 30% of the globally known diversity of grammitids. On average, we recorded 3.4 species and 117 individuals per plot. The maximum number of species recorded per plot was 18 in the Huancabamba region of Ecuador (America), 19 in the Arfak mountains of New Guinea (Asia), and 6 on La Réunion island (Africa) (Fig. 3).

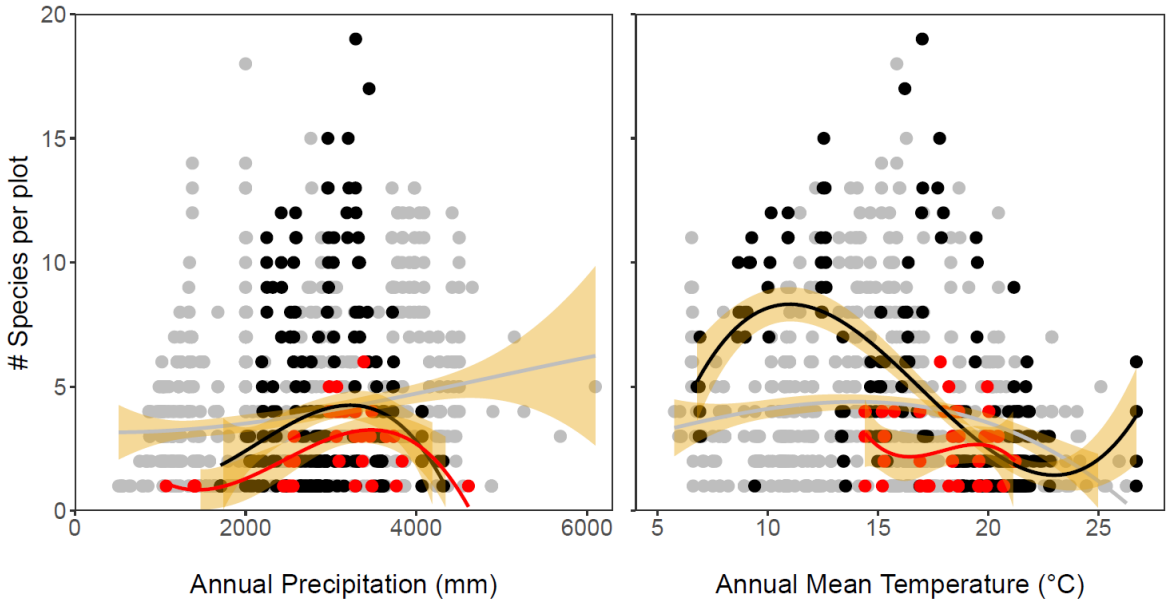

Figure 2. Number of species per plot in relation to mean annual temperature and precipitation. Dots representing plot values and trend lines are shown for America (gray), Asia (black), and Africa (red). Trend lines were fitted using a spline method with 95% confidence intervals (yellow). The distribution of the number of species per plot along both gradients did not differ between the three regions (Table 2).

Although the three regions differed in their climatic conditions (mean annual precipitation: R^2= 0.01546, F=18.08, p-value=2.296^{e-05}, mean annual temperature: R^2=0.09207, F=111.3, p-value= < 2.2^{e-16}), the number of species per plot along the gradients of precipitation and temperature was not significantly different between the three continents (Table 2). The distribution of species along the climatic gradients showed that in the three continents the highest richness occurred in areas of average mean annual precipitation values above 2000 mm and temperatures below 20°C (Fig. 2).

Table 2. Multiple regression analyses for the distribution of species richness per plot along the gradients of temperature and precipitation within each region. Region was included as a covariate to account for the differences in the climatic conditions between continents.

Temperature*Precipitation*Region	Estimate	Std. error	t value	p value
America vs Africa	-9.129^{e-05}	2.286^{e-04}	-0.399	0.69
America vs Asia	1.509^{e-04}	9.118^{e-05}	1.654	0.10
Asia vs Africa	-3.334^{e-04}	4.363^{e-04}	0.764	0.45

The regression analysis showed that in both America and Asia the relationship between the number of species and the number of individuals per plot was better described by a logarithmic model than a linear or quadratic one (Fig 3, only the two better fits are shown). As such, the increase in the number of individuals initially increased proportionally with the increase in the number of species per plot up to a certain limit where the relationship decelerated and did no longer describe a proportional increase.

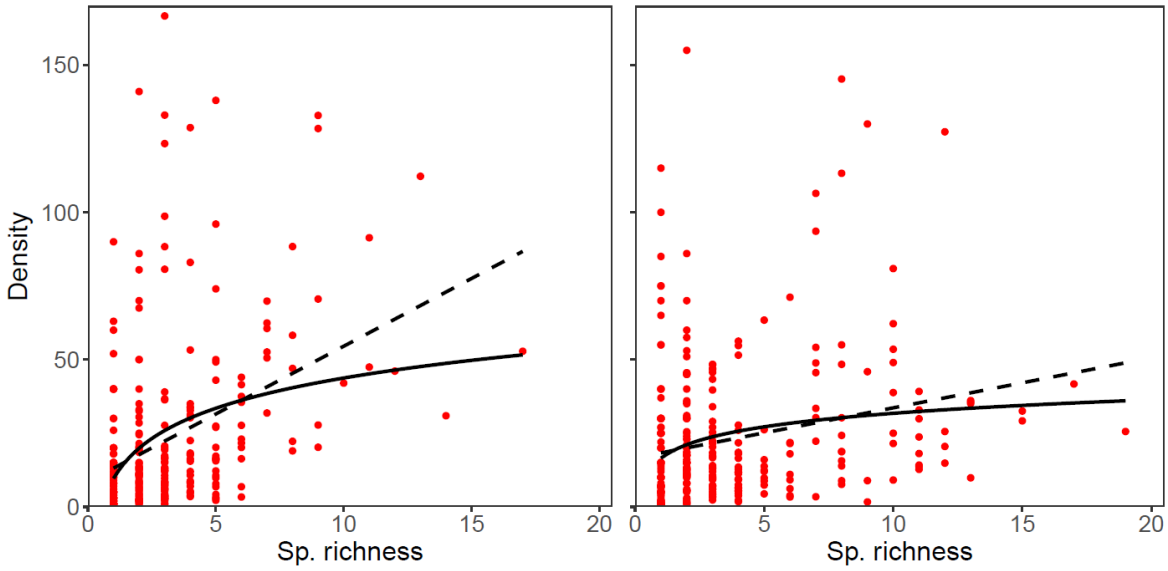

Figure 3. Relationship between the average number of individuals per species per plot (density) versus the total number of grammitid species per plot (richness) in 296 plots in America (a) and 307 plots in Asia (b). The logarithmic lines are better fits to the empirical data than the linear relationships, indicating niche limitation. Left panel: America, linear model: AIC=1353.368; Adj R^2= 0.057; p-value= 1.737^{e-05}; log model: AIC=602.368; Adj R^2=0.0634; p-value= 6.893^{e-06}. Right panel: Asia, linear model: AIC=1605.941, Adj R^2= 0.0331; p-value= 0.0008; log model: AIC= 733.773; Adj R^2= 0.025; p-value= 0.00325.

Discussion

Our results suggest that the number of coexisting grammitid species per plot was similar under comparable climatic conditions on the three continents. Considering that the continents have a different number of species and underlying diversification histories and rates (Table 1), this is indicative of spatial stability in species richness among assemblages on the three continents. Even in Africa, which only has about 10% of the richness of grammitids of the other continents, local richness at the plot level was only slightly lower (and non-significantly so) than in America and Asia. All this suggests that there may be an upper limit to the number of species that can co-occur in a single plot. This limit appears not to be directly set by the number of grammitid individuals that can occur in these plots because these small ferns occurred at high densities of on average 117 individuals per plot, which was over 30 times the average number of species.

On the other hand, we found that the increase in the number of species per plot in America and Asia does not result in the increase in the number of individuals present, providing indirect evidence of niche limitation. This stands in contrast to the common opinion that competition plays only a limited role in epiphyte communities because of the low densities of individuals and the highly dynamic nature of their habitat limit species interactions (Benzing 1990, Zotz and Hietz 2001, Zotz 2016). This would especially apply to grammitid ferns, which are small plants and typically grow well spaced from one another. In our case, the mean number of 117 individuals per plot translates into an average of one individual per about 3.5 m^2 ground surface. While this does not directly correspond to the surface area available on the tree trunks and branches, it nevertheless shows that these plants typically grow so far apart that they do not touch each other. So how could competition occur?

We see three possible explanations for this pattern. First, there are many other vascular epiphytic plants besides grammitid ferns in the forests studied by us (Krömer et al. 2005, 2007). Thus, competition may not occur mainly between the grammitid ferns themselves, but between all epiphytes. Second, competition between the fern species may occur primarily during the establishment phase. Ferns require specific, stable habitat conditions to go through the phases of gametophyte germination and growth, followed by fertilization and sporophyte development (Watkins et al. 2007). Suitable sites

for the full development of young ferns may be spatially and temporally restricted, so that the density of ferns is primarily determined at the recruitment phase, rather than at the stage of the mature plants. Similar conclusions on the crucial importance of the establishment phase in determining fern density have been drawn for terrestrial ferns(de Oliveira Rodrigues and Costa 2012, Kessler et al. 2014). A third possibility is that extreme habitat specificity explains the present pattern. According to Hutchinson (1957), if each species is limited by the number of territories that can be set up in a local area, even if all other requirements abound, it is possible that competition is entirely intraspecific. In this case, area is a limiting resource only if it is used by individuals of the same species and therefore, the distribution of species would be independent of each other.

Bringing our observations together, we conclude that there is preliminary evidence indicating that the number of species of grammitid ferns that co-occur at a local plot scale is at or approaching the level of ecological saturation. This is in contrast with the evidence at the regional scale, which shows no evidence of a slowdown in diversification rates (Sundue et al. 2015, Bauret et al. 2017). This apparent contradiction is in fact in line with ecological and evolutionary theory, which predicts that while there is a strong feedback between local and regional dynamics (Ricklefs 1987, 2004, Rabosky and Hurlbert 2015), different mechanisms operate at each scale (Cornell 1999, Harmon and Harrison 2015) and therefore their biodiversity patterns must not necessarily be synchronised. At the regional scale, grammitid diversification rates have been influenced by the availability of new habitat types mediated by geological and paleoclimatic events (Haufler et al. 2000, Kreier et al. 2008, Sundue et al. 2015), whereas at the local scale composition and structure of grammitid communities appears to be driven by climatic conditions, specially by changes in temperature and water availability along the vertical-epiphytic gradient (Zotz 2016).

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EDUCATION

06.2014–Present	PhD Ecology. University of Zurich. Zurich, Switzerland. Advisor: Dr. Michael Kessler, advisor)
09.2011–08.2013	MSc Ecology *cum laude*. VU University Amsterdam. Amsterdam, The Netherlands. Thesis title: Long-term surveillance of palm community composition in the western Amazon basin. Advisors: Prof. Dr. Henrik Balslev and Prof. Dr. Peter M. van Bodegom.
08.2004–03.2010	BSc. Biology. National University of Colombia. Bogotá, Colombia. Advisor: Prof. Dr. Gloria Galeano.
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EMPLOYMENT HISTORY

06.2014–Present	PhD Ecology. University of Zurich. Zurich, Switzerland. Advisor: Dr. Michael Kessler.
11.2010–04.2011	Internship: "Spatially explicit modelling of the spread of plant seeds". Department of Ecology and Geobotany, Institute of Ecology, Evolution and Diversity. Goethe University. Frankfurt am Main, Germany. Advisor: Prof. Dr. Oliver Tackenberg
01–05.2010	Research assistant: "Ethnobothany of Ñames (Dioscoreaceae) in Colombia". Institute of Natural Sciences. Advisor: Dr. Lauren Raz
01.2007–12.2008	Administrative assistant: "Biodiversity Informatics" Institute of Natural Sciences. National University of Colombia. Advisor: Dr. Lauren Raz
06.2005–12.2007	Student assistant: Colombian National Herbarium (COL). Bogotá, Colombia. Advisor: Prof. Dr. Gloria Galeano

APPROVED RESEARCH PROJECTS
National Geographic Society- Waitt Grant No.W468-16. "Biocultural Diversity of the Sierra Nevada del Cocuy: towards a UNESCO World Heritage Site".

SUPERVISION OF STUDENTS

04.2016–03.2017	MSc student at UZH: Anna Glanzmann. Thesis project: "The role of microhabitat segregation in the coexistence of grammitid ferns (Polypodiaceae)"

TEACHING ACTIVITIES

Fall semester 2016	Teaching assistant in BIO227 "Biogeography and Biodiversity" MSc course UZH
Fall semester 2016	Teaching assistant in BIO116 "Phylogeny and Adaptation" BSc course UZH

MEMBERSHIPS

PhD student representative at the European Network of Palm Scientist (EUNOPS), Sociedad Colombiana de Botánica, International Biogeography Society (IBS), Society for Tropical Ecology (GTÖ), Grupo de Investigación de Palmas Silvestres Neotropicales.

ORGANISATION OF CONFERENCES

06.2015	Co-organizer of the World Palm Symposium 2015 & Organizer of the Publication Strategies Workshop. http://palms2015.au.dk/publication strategies-mini-workshop/

AWARDS & FELLOWSHIPS

VU University Amsterdam-Fellowship Programme: 29,000 €; Alberta Mennega Stichting: 1,000 €; VU University Amsterdam-Fondsendesk scholarship: 300 €; Department of Bioscience-Aarhus University: 29,000 DKK. Graduate Campus (GRC) Travel Grants, University of Zurich: 2,800 CHF, Swiss National Science Foundation: 83,650 CHF.

PERSONAL SKILLS

Languages:	Native Spanish, fluent English, basic German
Software:	Multivariate Analysis in R, Applications in GIS and Remote Sensing (ArcGIS, SAGA, QGIS), Community Ecology Analysis in Estimate*S*
Fieldwork:	Chile-Patagonia 2018 (1 month); Colombia-Andes 2017 (1 month); Costa Rica 2016 (1 month); Colombia-Amazon & Andes 2015 (2 months); Ecuador-Andes 2014 (1 month); Peru-Andes & Amazon 2014 (2 months); Bolivia-Andes 2014 (2 weeks); Colombia-Caribbean 2014 (3 weeks); Bolivia-Andes 2013 (3 weeks); Ecuador-Amazon 2012 (1 month); Colombia-Pacific 2011 (2 weeks), Colombia-Pacific 2010 (1 month); Colombia-Andes 2009 (3 months); Colombia -Magdalena Valley 2008–2009 (6 months); Colombia-Eastern Plains 2007–2008 (3 months).

RESEARCH STAYS

05.2015	New York Botanical Garden, USA. Taxonomy of the palm genus *Geonoma*. Advisor: Dr. Andrew Henderson
09–12.2013	Ecoinformatics and Biodiversity Group. Aarhus University, Denmark. Effects of climate change in Amazonian forests. Advisors: Prof. Henrik Balslev, Prof. Jens Christian Svenning

PEER-REVIEWED PUBLICATIONS

Olivares, I., D.N. Karger & M. Kessler. 2018. Assessing species saturation: conceptual and methodological challenges. *Biological Reviews*, doi: 10.1111/brv.12424.

Sundue, M, **I. Olivares** & M. Kessler. 2018. Ascogrammitis lehnertii (Polypodiaceae): A new species from a species-rich community of grammitid ferns in the Andes of Ecuador. *Systematic Botany* 43(3): pp. 709–716. DOI 10.1600/036364418X697427.

Pérez-Escobar, O.A., Cámara-Leret, R., Antonelli, A., Bateman, R., Bellot, S., Chomicki, G., Cleef, A., Diazgranados, M., Dodsworth, S., Jaramillo, C., Madriñan, S., **Olivares, I**., Zuluaga, A. & Bernal, R. 2018. Mining threatens Colombian Ecosystems. *Science* 359, 1475.

Olivares, I. & Balslev, H. 2016. Gloria Galeano (22.4.1958–23.3.2016). *Botanical Journal of the Linnean Society* 182: 204–206. doi:10.1111/boj.12461

Olivares, I., J.-C. Svenning, P.M. van Bodegom, R. Valencia & H. Balslev. 2016. Stability in a changing world – palm community dynamics in the hyperdiverse western Amazon over 17 years. *Global Change Biology* 23 (3): 1232–1239, doi: 10.1111/gcb.13494

Olivares, I., J.-C. Svenning, P.M. van Bodegom & H. Balslev. 2015. Effects of warming and drought on the vegetation and plant diversity in the Amazon Basin. *The Botanical Review* 81(1): 42–69. http://link.springer.com/article/10.1007%2Fs12229-014-9149-8

Olivares, I. & G. Galeano. 2013. Leaf and inflorescence production of the wine palm (Attalea butyracea) in the dry Magdalena River Valley, Colombia. *Caldasia* 35(1): 37–48. http://www.ciencias.unal.edu.co/unciencias/data-file/user_16/file/caldasia/3501/cld350104.pdf

Bernal, R., G. Galeano, N. García, **I. Olivares** & C. Cocomá. 2010. Uses and perspectives of the wine palm, Attalea butyracea, in Colombia. *Ethnobotany Research & Application*. 8: 255–268. www.ethnobotanyjournal.org/vol8/i1547-3465-08-255.pdf

BOOK CHAPTERS

Ledezma-Rentería, E., Copete, J.C., Núñez-Avellaneda, L.A., **Olivares, I.** & G. Galeano. 2016. Biología poblacional y reproductiva, usos y manejo de la palma cabecinegro (Manicaria saccifera) en los bosques inundables del Chocó, Colombia. En: CA Lasso, G Colonnello, M Moraes (Eds). Morichales, Cananguchales y Otros Palmares Inundables de Suramérica, Parte II. Instituto Humboldt Colombia. https://www.researchgate.net/publication/311742153_La_palma_cabecinegro_Manicaria_saccifera_en_los_bosques_inundables_de_Choco_Colombia_biologia_poblacional_y_reproductiva_usos_y_manejo

Galeano, G. & **I. Olivares**. 2013. Palma de vino (Attalea butyracea). En: R. Bernal & G. Galeano (Eds.) Cosechar sin destruir. Aprovechamiento sostenible de palmas colombianas. Editorial Universidad Nacional de Colombia. Bogotá. Facultad de Ciencias. Instituto de Ciencias Naturales: PALMS: Colciencias. ISBN: 978-958-761-611-8.
http://www.fp7-palms.org/images/FILES/products/deliverables/del%2010/Cosechar_sin_destruir.%20completo.pdf

INTERNATIONAL MEETINGS

Oral presentations

08.2018 Assessing Species Saturation: Conceptual and Methodological Challenges. New Orleans, USA.

12.2016 A unified definition of community saturation. British Ecological Society Meeting. Liverpool, UK.

06.2015 Setting the Limits of Community Saturation. World Palm Symposium 2015. Quindío, Colombia.

05.2014 Limits and constraints of species radiations: Geonoma as a study case. European Network of Palm Scientists (EUNOPS) Meeting. Valencia – España,

09.2013 Monitoreo a largo plazo de una comunidad de palmas en la Amazonía occidental. International Symposium. Palm Harvest Impacts in Tropical Forests: Results and Proposals. Tingo Maria–Peru.

05.2013 Long-term change in palm community composition in the western Amazon basin. European Network of Palm Scientists (EUNOPS) Meeting. Aarhus– Denmark.

11.2012 Long-term change in a palm community in the western Amazon. Paleoecology and Landscape Ecology progress meetings. Instituut voor Biodiversiteit en Ecosysteem Dynamica (IBED), Universiteit van Amsterdam. Amsterdam–The Netherlands

Posters

04.2015 17 years of stability in a western Amazonian palm community. Olivares, I, J-C. Svenning, P.M. van Bodegom, R. Valencia & H. Balslev. “Annual conference of the Society for Tropical Ecology. Resilience of Tropical Ecosystems: Future challenges and opportunities” Zurich–Switzerland.

08.2013 Monitoreo a largo plazo de una comunidad de palmas en la Amazonía occidental. Olivares, I, J-C. Svenning, P. M. van Bodegom, R. Valencia & H. Balslev. “VII Colombian Botanical Congress”. Ibague–Colombia.

05.2010 Leaf production and phenology of Attalea butyracea in the dry Magdalena River Valley, Colombia. Olivares, I. & G. Galeano. “PALMS 2010 international Symposium on the Biology of the Palm family”. Montpellier–France, May 5–7, 2010.

04.2009 Efectos de la sequía en la fisiología del crecimiento de Phaseolus vulgaris var Agrario. Olivares, I. & D.G. Zambrano. “V Colombian Botanical Congress”. Pasto–Colombia, April 19–24, 2009.

OUTREACH ACTIVITIES

02.2013–Present Member of the initiative: Sierra Nevada del Cocuy, Colombia towards a UNESCO

World Heritage Site. www.cocuyworldheritage.org/.

THESES

Master: Long-term surveillance of palm community composition in the western Amazon basin. Vrije Amsterdam University, The Netherlands. Completed July-2013. http://www.fp7-palms.org/images/FILES/products/theses/THESES/MSc_Theses_PALMS/OLIVARES_2013_MSc_Thesis_AMSTERDAM.pdf

Bachelor: Producción de hojas y fenología de la palma de vino (Attalea butyracea) en el valle seco del río Magdalena, Colombia. Universidad Nacional de Colombia, Bogota. Completed 2009. http://www.fp7-palms.org/images/FILES/products/theses/THESES/1st_Degree_Theses_PALMS/OLIVARES_2009_Pregrado_Thesis_ICN_BOGOTA.pdf